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PREFACE

A symposium on ''the Chemistry of Natural Products'' was organised on the occasion of the thirty third annual meeting of the National Academy of Sciences India, held at Allahabad on Feb. 15 and 16, 1964. About forty workers in the field participated in the symposium.

After the introductory remarks by Dr. J. D. Tewari of the Allahabad University, the first invited lecture on ''the Stereochemistry of Carvomenthols and Related Compounds'' was delivered by Dr. G. S. Siddhu, Director, Regional Research Laboratory, Hyderabad and this was followed by discussion of the contributed research papers. Another invited lecture on ''the Chemistry of the Components of Certain Indian Essential Oils'' was delivered by Prof. S. S. Deshpande from Indore. Dr. S. C. Pakrashi of the Indian Institute for Biochemistry and Experimental Medicine, Calcutta delivered a lecture on ''Application of Mass Spectrometry in the Structual Determination of Alkaloids.'' These three lectures and the twenty contributed papers are being published in this issue of the Prceedings of the Academy.

As the convenor of the symposium, I take this opportunity to express our grateful thanks to the participants for their contributions and co-operation and to all others who helped to make the symposium a success.

Chemistry Department,
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R. D. Tiwari
Convenor

Nov. 1, 1965

STEREOCHEMISTRY OF CARVOMENTHOLS AND RELATED COMPOUNDS

By

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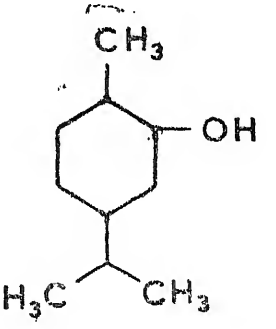
SUMMARY

The conformations of the 4 stereoisomeric carvomenthols were studied by Johnston and Read in 1934 when rules of conformational analysis were not well established or understood. Later workers have used these assignments in correlating other terpenoids and therefore have arrived at some incorrect conformational assignments. In this paper the inconsistencies of Johnston and Read's work are pointed out and the necessity to assign the correct conformation to the isomeric carvomenthols and related compounds is emphasised.

Carvomenthol (I) has three asymmetric carbon atoms and can, therefore, exist in four enantiomeric pairs.



I. Carvomenthol

The conformations of these as known to date are given in Table 1.

TABLE 1

Stereochemical Relationship of the Isomeric Carvomenthols

Isomer	Steric Relationship of Substituents at Asymmetric carbon Atoms			Conformation		
	C—1, C—2	C—2, C—4	C—1, C—4	C—1	C—2	C—4
(±)—Carvomenthol	*trans*	*cis*	*trans*	e	e	e
(±)—*Iso*carvomenthol	*cis*	*cis*	*cis*	a	e	e
(±)—*Neo*carvomenthol	*cis*	*trans*	*trans*	e	a	e
(±)—*Neoiso*carvomenthol	*trans*	*trans*	*cis*	a	a	e

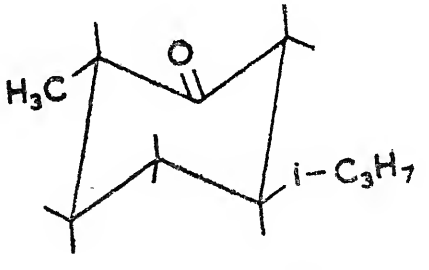
The assignment of these conformations has involved an approach similar in many respects to that of the menthols.[1] In the case of monthols, the starting compound has been piperitone which was hydrogenated to yield isomeric menthones and menthols. The parent compound from which the carvomenthols and the related compounds have been derived is (+)—carvone.[2] Johnston aud Read[2–4] were the first to investigate the stereochemical aspects of these alcohols. Later results have been based on their findings and hardly any further work on stereochemistry of carvomenthols has been reported.

Johnston and Read have made three approaches to this problem: (i) the hydrogenation of carvone to carvomenthones and their reduction to carvomenthols,[3] (ii) conversion of isomeric carvomenthones to respective oximes amines and their deamination to corresponding carvomenthols[3], and (iii) reduction of carvone to carveols and their hydrogenation to saturated alcohols.[3,4]

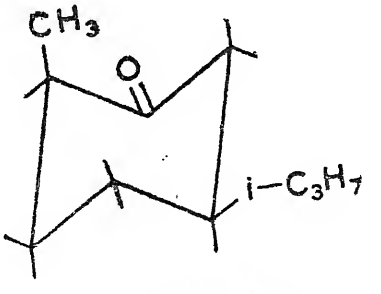
A closer examination of the results obtained from the above shows that they do not always correspond to the established principles of conformational analysis. Also, there are some inconsistencies which remain to be explained. These aspects will be described here briefly and the discrepancies pointed out.

(i) The isomeric carvomenthones were obtained by the catalytic hydrogenation of (+)—carvone[2]. It has been found that the carvomenthones thus obtained have about 60 per cent of the more stable isomer carvomenthone (II) which has been assigned the *trans* (1e, 4e) conformation. The other isomer with higher refractive index and density and also having a higher numerical rotatory power, on the basis of the Auwers-Skita rule[5,6] and in keeping with the menthone series has been designated as *iso*-carvomenthone (III) and assigned the *cis* conformation (1a, 4e)[3]. In an equilibrium mixture, carvomenthone is present to about 80 per cent.

The configurations of the isomeric carvomenthones have later been independently established by McNiven and Read[7] in 1952. They converted the crystalline



II. Carvomenthone

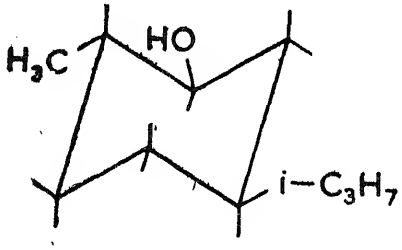


III. Isocarvomenthone

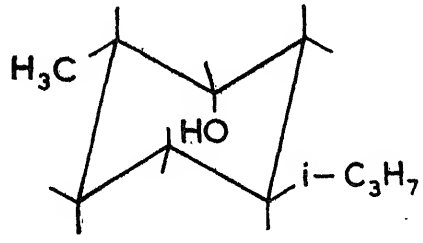
carvomenthone oxime to carvomenthylamine and this to carvomenthyl trimethylammonium hydroxide. On decomposing the latter only *trans-p*-menth-2-ene was obtained, which shows the *trans* conformation for the carvomenthone.

(—)-Carvomenthone on reduction with aluminium isopropoxide is reported to have given two carvomenthols[3]. A carvomenthol with a higher rate of esterification has also been obtained by the sodium and alcohol reduction of carvomenthone. This reduction is known to lead preferentially to the thermodynamically more stable isomer[15] and so the resultant carvomenthol will be expected to have all the

three substituents equatorially bound. This has been named as carvomenthol (IV). The other isomer from the Meerwein-Ponndorf's reduction of carvomenthone will be expected to differ from carvomenthol only with respect to the conformation of the hydroxyl group, which will be axial. This isomer has been called *neo*-carvomenthol



IV. Carvomenthol



V. Neocarvomenthol

(V) after the nomenclature in menthols. The stereochemical aspects of the reduction of *iso*-carvomenthone directly to *iso*-carvomenthols do not appear to have been studied.

(ii) The carvomenthone mixture obtained by the reduction of (+)-carvone was converted to oxime[2]. Carvomenthone oxime was separated as a crystalline solid while *iso*-carvomenthone oxime was obtained as a syrupy liquid.

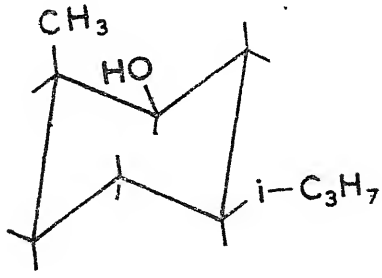
On reducing carvomenthone oxime with sodium and alcohol,[3] two carvomenthylamines were obtained which on purification were identified as carvomenthylamine (92·5%) and *neo*-carvomenthylamine (7·5%) on the basis of their deamination reactions, besides the fact that the sodium and alcohol reduction of an oxime also yields predominantly the stabler isomer having the amino group in the equatorial conformation.[15]

It is known that on deamination with nitrous acid, an equatorial amino group is almost exclusively converted to an equatorial hydroxyl group while an axial amino group yields mostly a terpene together with a mixture of epimeric alcohols in which the conformer with the equatorial hydroxyl dominates[8,9]. The identity of the alcohols resulting by the deamination of carvomenthylamine and *neo*-carvomenthylamine was also established with carvomenthol and *neo*-carvomenthol. On oxidation with Beckmann mixture, carvomenthol and *neo*-carvomenthol have yielded carvomenthone, which supports their assignments.

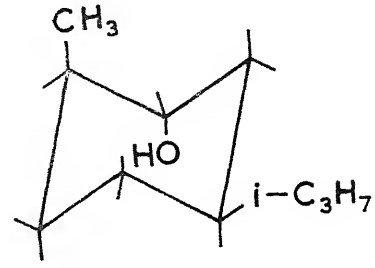
McNiven and Read[7] have carried out the decomposition of carvomenthyltrimethylammonium hydroxide. According to Dhar et al.[10] in such bimolecular E_2 reactions, a *trans*-elimination takes place. The exclusive formation of *trans*-*p*-menth-2-ene from carvomenthylammonium hydroxide can occur only if the quaternary ammonium group has an equatorial configuration. It follows that the amino and the hydroxyl groups in carvomenthylamine and carvomenthol respectively must have the same configuration. *Neo*-carvomenthol obtained from the reduction of carvomenthone oxime must be the epimeric form.

The syrupy *iso*-carvomenthone oxime was also reduced to *iso*-carvomenthylamine and the deamination of the amine gave an alcohol together with some terpene[3]. The alcohol has been designated as *iso*-carvomenthol and Bose[11] has assigned it the aee conformation (VI).

Neoiso-carvomenthylamine has not been isolated and its deamination reaction has therefore not been studied. Bose[11] has assigned the corresponding alcohol the aae conformation (VII).



VI. Isocarvomenthol



VII. Neoisocarvomenthol

(iii) The third approach to prepare and identify carvomenthols is through the intermediate formation of carveols from (+)-carvone[4]. It is here that the discrepancies from the already assigned conformations are observed.

Johnston and Read[4] reduced (+)-carvone to isomeric carveols using aluminum isopropoxide. *Cis*- and *trans*- carveols were separated as 3,5-dinitrobenzoates and the pure carveols were obtained from them. In keeping with the Auwers-Skita rule[5,6] they assigned the *cis* configuration to the carveol which had the higher physical constants. It is now known that this rule is reserved in the case of 1,3 disubstituted cyclohexane systems[12]. The *cis* conformer in 1,3-disubstituted cyclohexane systems with both the substituents preferably in the equatorial position will have a low energy content and will be more stable. The less stable *trans* conformer with hydroxyl group in the axial position will have a higher energy content and thus will be expected to have a higher refractive index and density in accordance with the well-established rules.[13,14]

Further, Johnston and Read found that the *trans*-carveol had a higher rate of estrification (1·57 times more)[4]. It is well known that an equatorial and not an axial hydroxyl is esterified or the resulting esters hydrolyse more readily.[15] On this basis also, the assignments to the isomeric carveols appear to be incorrect.

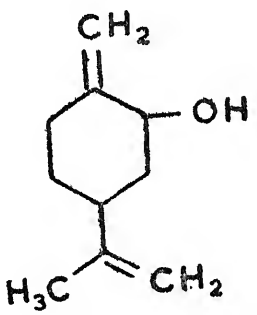
The palladium-catalysed hydrogenation of *cis*-carveol by Johnston and Read[3] led to he formation of carvomenthol and *neoiso*-carvomenthol. Similarly, the hydrogenation of *trans*-carveol gave a mixture of *neo*-carvomenthol and *iso*-carvomenthol. Assuming the assignments of Johnston and Read to carveols to be correct it would appear that in both cases in addition to an expected hydrogenation product another isomer is formed which can arise only by the epimerisation at the hydroxyl carbon atom. Such epimerisations under the conditions of hydrogenation have not been reported and it follows that the only other way to explain these results is to consider the earlier conformational assignments to carvomenthols as incorrect. It will also be noted that even on inverting the conformational assignments for carveols, the assigned conformations to the carvomenthols cannot be explained.

Apart from Johnston and Read's, no significant work has been carried out in this direction. Their work dates back to about 1934, when the concept of conformational analysis was not developed. Also, many of the stereospecific

reaction which now have almost become the general rules for the assignment of conformations to the molecules, were not known. With the advances in conformational analysis during recent years a detailed study of the isomeric carvomenthols and related compounds will, therefore, be of great interest. The known general methods for the determination of the conformation, both physical and chemical can now be employed to advantage in this respect.

This is further important because the later workers have correlated their results on the assignments proposed by Johnston and Read and thus have often come to wrong conclusions. Thus, Blumann et al.[16] in elucidating the conformations to isomeric *p*-menth-1-ene-3,6-diols have hydrogenated the respective esters to corresponding menthol and carvomenthol isomers. Reducing *p*-menth-3-toluene-*p*-sulphonate-6-3,5-dinitrobenzoate by lithium aluminium hydride they obtained *iso*-carvomenthol and on this basis assigned an equatorial conformation to the hydroxyl group at C-6. They also obtained *iso*-carvomenthol together with *neo*-carvomenthol by catalytic hydrogenation of *trans* carvotanacetol. The last compound should have the hydroxyl group axially bound and will not be expected to yield a product with an equatorial hydroxyl group. This also points to an incorrect assignment to carvotanacetols and/or to carvomenthols resulting in a false assignment to *p*-menth-1-en-3,6-diols.

Similarly, during the chemical examination of the essential oil of *Cymbopogon densiflorus*, Naves and Grampoloff[17] came across a new alcohol, (+)-*p*-mentha-1 (7), 8-dien-2-ol (VIII) to which they assigned the *cis* conformation (OH equatorial) on the basis of its hydrogenation to supposed *iso*-carvomenthol. They also showed the



VIII. *p*—Mentha-1 (7) ; 8-diene-2-ol

presence of this alcohol in the essential oil of *Cymbopogon martini*, var. sofia with the help of gas-liquid chromatography and fractionation data.

We have also carried out a detailed chemical examination of gingergrass oil (*Cymbopogon martini*, var. sofia) and have identified the same alcohol.[18] This, however, under the conditions of hydrogenation reported by Naves and Grampoloff[17] (Pt (O_2)/ EtOAc) gave predominantly carvomenthol.

We also assigned the above alcohol a *cis* conformation. It was noted that not only were the physical constants of our purified alcohol different from those reported by Naves and Grampoloff but also the melting point of the 3,5—dinitrobenzoates of the two alcohols showed a difference of about seven degrees. We presumed the alcohol reported by Naves and Grampoloff to be the *trans* isomer as the refract-

ive index and the density of their alcohol were higher than those observed by us. The anamolies in the conformational assignments of carvomenthols seem to have led Naves and Grampoloff[17] to identify the hydrogenation product of the *p*-mentha-1(7),8-dien-2-ol as *iso*-carvomenthol which actually should be an isomer of the *neo* series (axial hydroxyl). Later work of Klein and Ohloff[19] on the epoxidation of carbonyl compounds in monoterpenoids and that of Schroeter[20] on the hydroperoxidation of limonene supports our presumption. The alcohol which Naves and Grampoloff isolated should be designated *trans* and the one isolated by us in the *cis*.

These examples are enough to demonstrate the necessity of having the correct conformational assignments for carvomenthols and other related compounds.

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CHEMISTRY OF THE COMPONENTS OF CERTAIN INDIAN ESSENTIAL OILS

By

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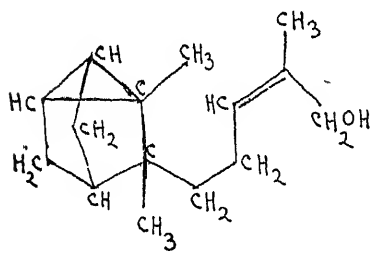
ABSTRACT

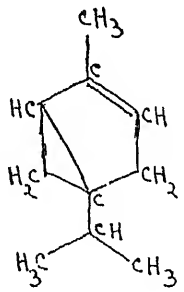
Some Indian essential oils are conspicuous due to their main components possessing a cyclopropane ring stucture. The stabilities of a double bond, a cyclopropane ring and a cyclohexane ring occurring in one and the same molecule are compared. The application of Markownikoff's rule to addition reactions at a double bond and a cyclopropane ring is critically examined.

By the term "Indian essential oils" is meant here those oils of Indian origin whose components have a special feature. The speciality of feature is either (a) a molecular structure which is rarely found in components of oils of other origins or (b) the very high proportion in which the component occurs in the oil. For example sandal wood oil is a typical Indian essential oil, because its main component α—Santalol has in its molecule a cyclopropane ring fused on to a cyclohexane ring- a structure which is unique. Again Rosha grass oil is a typical Indian essential oil in which geraniol occurs to the extent of ninety percent and even more. In this lecture the speciality of feature of the component of the oil considered is its molecular structure.

The fusion of a cyclopropane ring with a cyclohexane ring which is the structural speciality of α- santalol (I) is also noticed in α-thujene (II), Δ^3 carene (III) and Δ^4 carene (IV). α Thujene is the chief component of the essential oil derived



(I)



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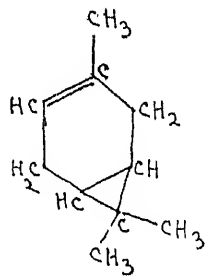
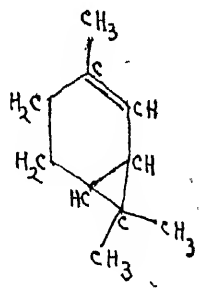


(III)



(IV)

from the gum-oleo-resin of Boswellia serrata Roxb. It constitutes about seventy per cent of the oil. Δ^3 carene is a component of the essential oil from the oleo-resin of Pinus longifolia of which it forms thirty seven per cent, while Δ^4 carene is a component of the essential oil from the grass Andropogon jwarancusa jones grown wildly in Uttar Pradesh. The exact proportion of Δ^4 carene in the oil does not appear to have been determined.

The cyclopropane ring is the smallest sing and has therefore according to Baeyer's strain theory the greatest strain. The occurrence of the cyclopropane ring in natural products is not therefore much expected. It is therefore surprising

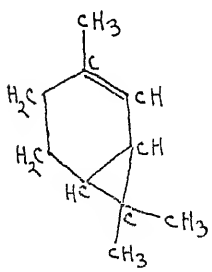
that the Indian essential oils such as those mentioned above should be very rich in components having in their molecules this unstable cyclopropane ring.

A striking speciality of the structures (I), (II), (III), and (IV) is that in addition to the cycle propane ring they have in them a cyclohexane ring and a double bond. The cyclohexane ring is quite stable, but the double bond is quite unstable in the sense that it easily opens up by addition producing a single bond or causing a rupture between the two carbon atoms which were held by it. The occurrence of these three structural elements, of varying degree of instability, in a single molecule has thus provided to the organic chemist an interesting field of study of addition reactions.

A closer inspection of the structures (I), (II), (III) and (IV) will show that in each of them the cyclopropane ring is fused to the cyclohexane ring. But whereas the double bond is outside these rings in (I), it is a part of the cyclohexane ring in (II), (III) and (IV), which consequently is affected by its presence.

Addition of the reagent HX to the structures (II), (III) and (IV) should therefore take place in the following sequence. The reagent will add to the double bond first as it is more strained than the cyclopropane ring. This will produce a saturated cyclohexane ring. Next it will add to the cyclopropane ring which will result in its opening. Thus finally the cyclohexane ring alone will survive. But there is also an alternative sequence of addition. The addition at the double bond started by the reagent may continue at the same site causing ultimately complete rupture of the double bond and therefore rupture of the cyclohexane ring. This will result in the survival of the cyclopropane ring alone finally.

Both these sequences of addition have been observed. Thus ethereal hydrogen chloride adds to Δ^4 carene producing the monohydrochloride (V). Acetic acid and hydrogen chloride crrry the process of addition further and produce dipentene dihydrochloride (VI). The cyclopropane ring has opened and the cyclohexane ring has survived.



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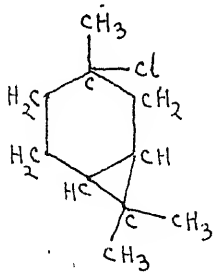

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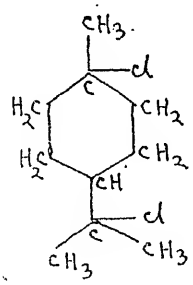

(VI)

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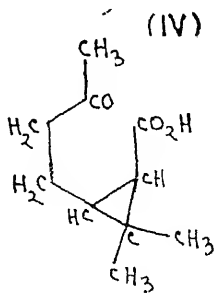
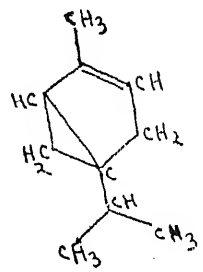


(VII)

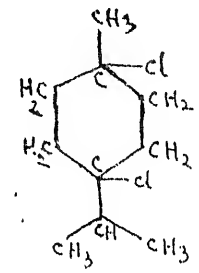
As an example of the alternative sequence of addition the oxidation of Δ^4 carene by acidified potassium dichromate may be mentioned. In this case the cyclohexane ring is ruptured with retention of the cyclopropane ring. The product of oxidation (caused by two successive additions at the double bond) is the keto acid (VII).

Application of Markownikoff's rule. Another interesting field of study for the organic chemist is to observe points of attachment between the addendum HX and the double bond or the cyclopropane ring. Markownikoff's rule in this connection tells us what is expected. According to this rule X (the negative component) of the reagent HX attaches to the carbon atom which is connected to the maximum number of carbon atoms, and H is connected to that carbon which is connected with the minimum number of carbon atoms. This rule is applicable to a double bond, in which only two carbon atoms are involved, and also to the cyclopropene ring in which three carbon atoms are involved.

The addition of hydrochloric acid to the double bond of Δ^4 carene (IV) to produce the monohydrochloride (V) and the addition of the same reagent to the cyclopropane ring in (V) to produce dipentene dihydrochloride (VI) are in accordance with the Markownikoff's rule. But in the formation of terpinene dihydrochloride (VIII) by the addition of hydrochloric acid to α- thujene, this rule is not obeyed as far as the addition to the cyclopropane ring as concerned. The rule requires formation of the structure (IX)



(II)



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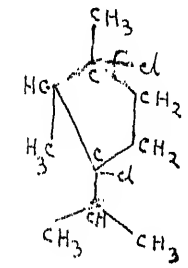
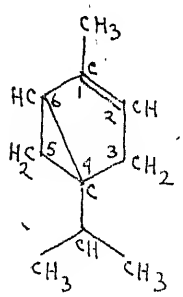


(IX)

In this connection an interesting observation about the action of nitrosyl chloride on *d*-α- thujene has been made at the chemistry laboratory of Holkar Science College Indore. A solid addition product, the thujene nitrosochloride has been isolated. This must have the structure (X). In addition to the solid nitrocochloride two oxidation products of the terpene were isolated These were identified as p. cymene (XI) and carvacrol (XII).



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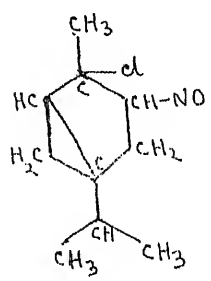
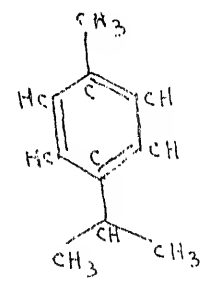
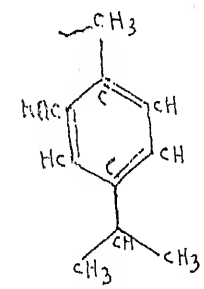


(X)

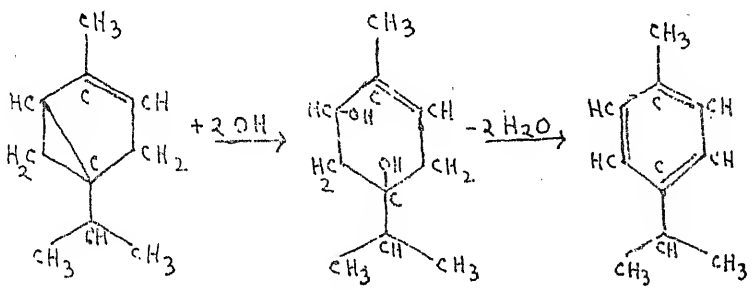


(XI)



(XII)

Nitrous acid is known to react in some reactions as an oxidising agent. The nitrogen appears as nitric oxide NO, and it is reasonable to assume that -OH group becomes available for oxidation. The formation of *p*-cymene (XI) from thujene may be reasonably assumed as a result of addition of two OH groups at the carbon atoms 4 and 6 of the cyclopropane ring of thujene. The carbon atom 4 is connected with the maximum number of carbon atoms. The negative OH group should therefore attach here in accordance with the rule. Another OH group (again a negative group) should attach at carbon 6 which is connected to smaller number of carbon atoms than 4 but a larger number of carbon atoms than 5. The addition of two OH groups at carbon atoms 4 and 6 of the cyclopropane ring will produce the structive (XIII). Loss of two molecules of water from (XIII) will give *p*-cymene (XI).



(II) (XIII) (XI)

The formation of carvacrol (XII) by further oxidation is not however easily explainable.

NEW COLOURING MATTERS FROM CASSIA TORA LINN,

By

R. K. SHARMA *and* P. C. GUPTA

Chemical Laboratories, University of Allahabad, Allahabad

ABSTRACT

Three new colouring matters obtained from the fat soluble brown deposit from the seeds of Cassia tora Linn have been found to belong to anthraquinone group of colouring matters. The three colouring matters have been provisionally named Tora colouring matter A, shining red-orange flakes m. p. 236. 38°; Tora colouring matter B dark-red crystalline compound m. p. 196-98°; and Tora colouring matter C, yellow crystalline substance m. p. 230-32°. The three colouring matters are definitely anthraquinone derivatives as anthracene is obtained by Zn-dust distillation of the colouring matter A while Zn-dust distillation either of the colouring matter B or the colouring matter C yields 2-methyl anthracene which is further confirmed by IR peaks characteristic of anthraquinone or its hydroxy derivatives at 686 cm^{-1}, 1678 cm^{-1} and 1660 cm^{-1} are given by the respective colouring matters. As regards the orientation of the substitutent groups in the different colouring matters, attempts have been made on the basis of characteristic colour reactions, some chemical tests and IR given by the individual colouring matters.

Cassia tora Linn (N. O. Leguminosae) is a wild growing shrub practically in all parts of India. The plant has been known to be highly medicinal,[1,2] mainly for skin diseases. Inspite of its reputed medicinal value, very scanty chemical literature has been described. Elborne[3] reported the presence of a glucoside and Jois and Manjunath[4] and Tewari and Gupta[5] have chemically examined the fatty oil from the seeds. Recently C. S. Narayan and S. Rangaswami have reported three coloured substances from the defatted seeds of cassia tora. S. Rangaswami[7] claims to have established the so-called identity of tora substane B and tora substance C isolated by him with non-rubrofusarin and rubrofusarin.

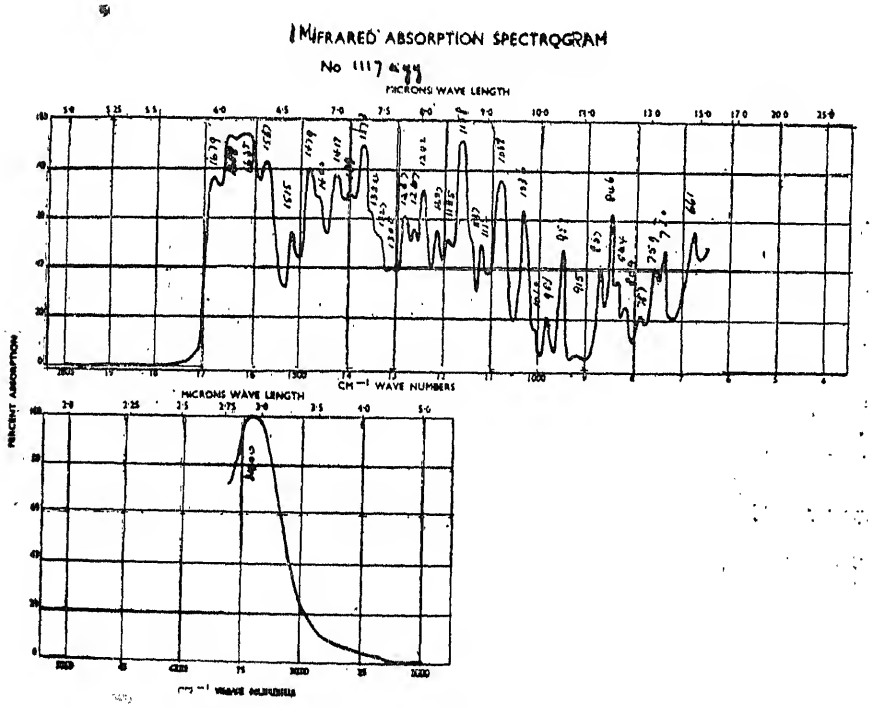
Authors have been able to isolate three colouring matters which being oil soluble are extracted in petroleum ether along with fixed oil and these are quite different from those obtained by S. Rangaswami (loc cit.). These are named tora colouring matter A, B and C. The three colouring matters belong to anthraquinone group of colouring matters as they give dark red colour in concentrated ammonia when warmed in presence of Zn-dust and on keeping turns yellow. Zn-dust distillation of the tora colouring matter A yields anthracene while B and C give 2-methyl anthracene. Presence of anthraquinone nucleus is further confirmed by the characteristic IR spectra of the three colouring matters A, B and C at 1686 cm^{-1}, 1678 cm^{-1} and 1660 cm^{-1} respectively.

Comparative data on some of the physical constants of the substances tora B ane C isolated by S. Rangaswami and the tora colouring matters B and C from cassia tora isolated by the authors and also of rubrofusarin and norrubrofusarin, are given in the following tables which indicate the clear distinction between the two types of colouring matters.

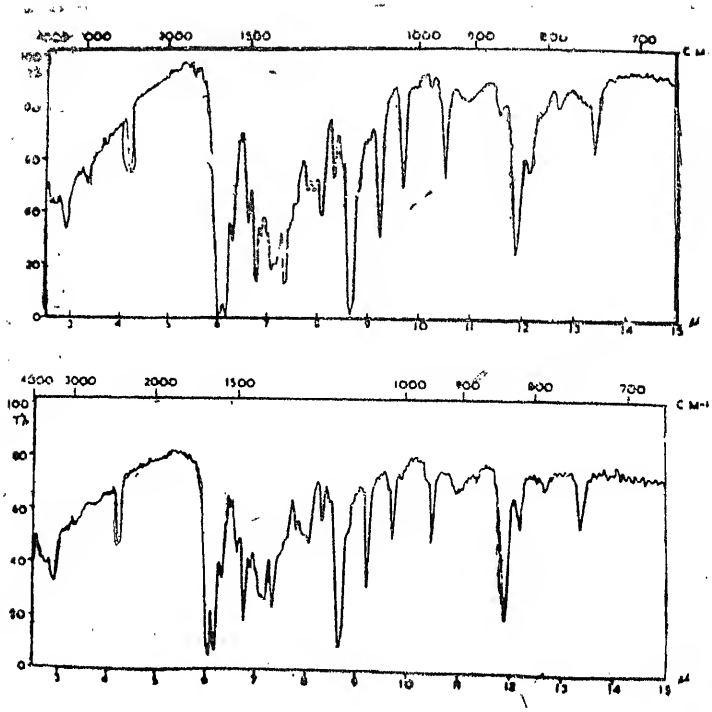
	Tora colouring matter B (Authors)	Tora C (S. Rangaswami)	Rubro fusrain
formula	$C_{16}H_{12}O_5$	$C_{15}H_{12}O_5$	$C_{15}H_{12}O_5$
colour	Dark-red	Red	Red
M. P.	196-98°C	208-09°C	210-11°C
M. P. diacetate	220-22°C	267-69°C	260°C
M. P. dimethyl ether	174° (decom.)	183-85°C	187-88°C

	Tora colouring Matter C (Authors)	Tora B (S. Ranga-swami)	Nor-rubro-fusrain
formula	$C_{15}H_{10}O_4$	$C_{14}H_{10}O_5$	$C_{14}H_{10}O_5$
colour	Yellow	Yellow	Yellow
M. P.	229-30°C	286-88°C (dec.)	280 (d)
M. P. diacetate	268-70°	206-09°	204°C
M. P. dimethyl ether	210° (decomp.)	207-09°C	203°C

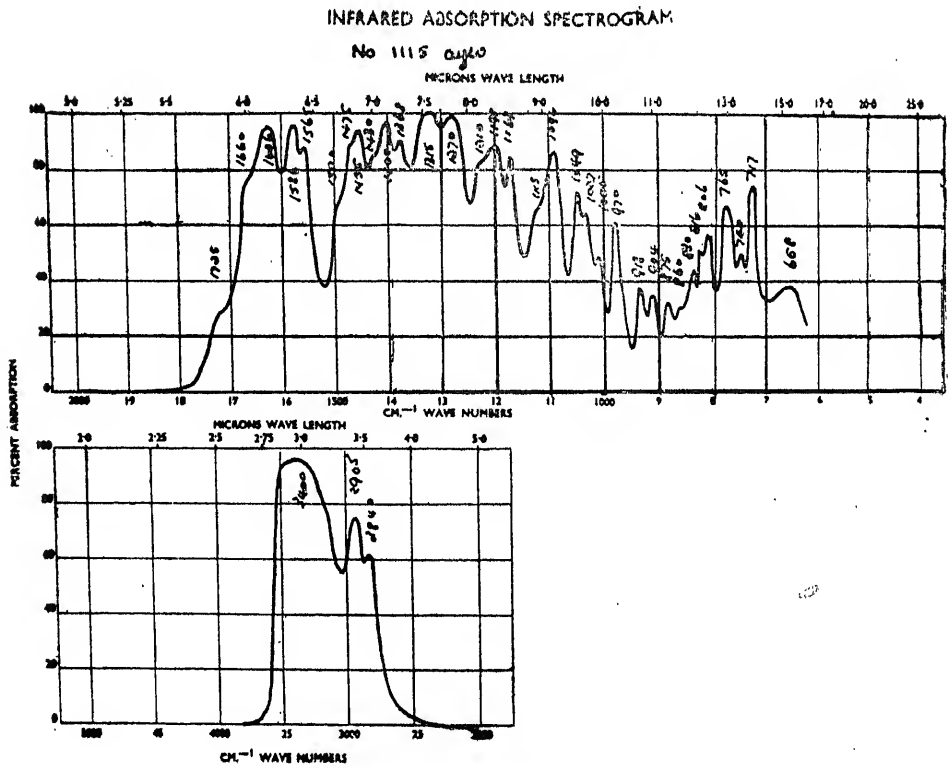
IR spectra are also given below where it can be noticed that there is no similarity in the two types of the colouring matters.



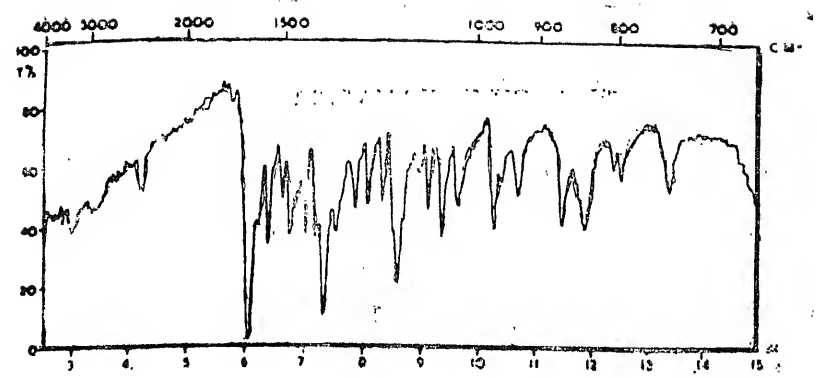
Tora Colouring Matter B. (authors).



No. 1. Tora C. (S. Rangaswami).
No. 2. Rubrofusarin.



Tora Colouring Matter C. (authors).



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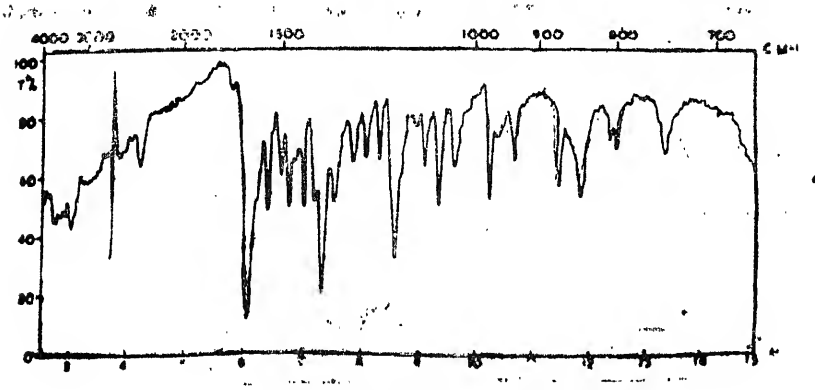

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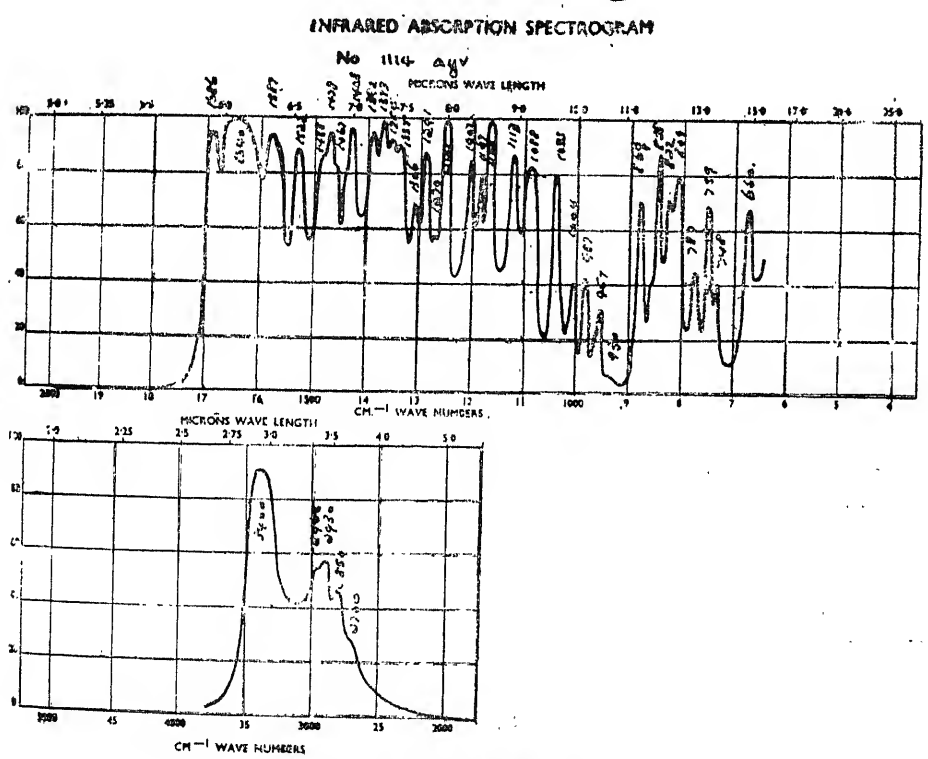
No. 3. Tora B. (S. Rangaswami)

No. 4. Nor-rubrofusarin.[7]

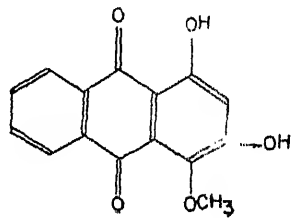


Tora Colouring Matter A. (authors).

TORA COLOURING MATTER A

Tora colouring matter A is shining orange-red leaflets m. p. 236-38°C having the molecular formula $C_{15}H_{10}O_5$ and gives a dirty green colour with ethanolic ferric chloride. It gives an intense red colour in concentrated ammonia in presence of Zn-dust and turns yellow on keeping. It contains two phenolic hydroxyl groups ond one methoxyl group. The zinc-dust distillation of the colouring matter A yields anthracene. It does not give flourescent solution in glacial acetic acid which eliminates the possibility of orientation of the hydroxyl groups in 1:4 position[8]. It dissolves slowly in aqueous sodium carbonate giving a yellow coloured solution. This property indicates that one of the two hydroxyl groups should be in β-position. With methanolic magnesium acetate an orange yellow colour is developed which is characteristic of the presence of two hydroxyl groups in 1:3-position in dihydroxy anthraquinones[9]. It decolourised alkaline potassium permanganate solution immediately and the recovery of phthalic acid only from this solution indicates that all the three substituted groups should be present in the same ring. Now there may be only two possible structures and one is already known in the literature.[10]

On the basis of the above experiments the following structure may be assigned to the tora colouring matter A.

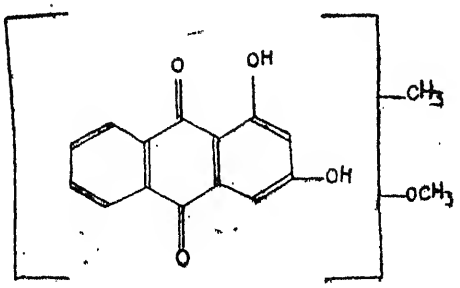


Tora Colouring Matter A

TORA COLOURING MATTER B

Tora colouring matter B is a dark red compound, m. p. 196-98°C, having the molecular formula $C_{16}H_{12}O_5$ and gives a blue green colour with ethanolic ferric chloride. An intense red colour is developed in concentrated ammonia in presence of little Zn-dust and on keeping turns yellow as in the case of A. Zn-dust distillation of the substance yields 2-methyl anthracene. It contains two phenolic hydroxyl groups and one methoxy group. It dissolves in aqueous sodium carbonate which indicates the presence of one free hydroxy group that is in β-position. With methanolic magnesium acetate an orange-yellow colour is developed which is a characteristic test colour reaction for 1:3-dihydroxy anthraquinone[9]. It does not give a fluorescent solution in glacial acetic acid which eliminates 1:4-positions for the two hydroxyl groups to be present in an anthraquinone. The I. R. absorption spectrum of the tora colouring matter B exhibits bands at 1679 cm^{-1} and 1628 cm^{-1} characteristic of an unco-ordinated carbonyl group and a carbonyl group co-ordinated with α-hydroxy group respectively in anthraquinones.[10] Orientation of the other two groups *i. e.* one methyl group and one methoxy group is still unsettled,

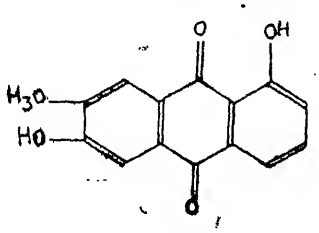
Thus the following structure may be assigned to tora colouring matter B.



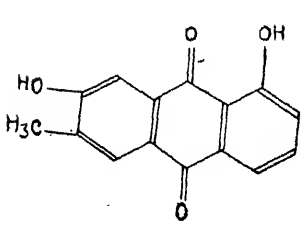
Tora Colouring Matter B

TORA COLOURING MATTER C

Tora colouring matter C is a yellow crystalline compound m. p. 229-30°C having the molecular formula $C_{15}H_{10}O_4$ and gives blue green colour with ethanolic ferric chloride. It also shows the colour reactions in concentrated ammonia in presence of little Zn-dust as in the case of the two colouring matters. The zinc-dust distillation of the compound yields 2-methyl anthracene and it contains two hydroxyl and one methyl group. With methanolic magnesium acetate a pink colour is developed which is a characteristic reaction for 4:5:7 tri-hydroxy anthraquinones[9]. It does not give a fluorescent solution in glacial acetic acid which is negative test for the presence of two hydroxyl groups in 1:4-positions. It dissolves instantaneously in aqueous sodium carbonate, this property indicates that one of the two hydroxy groups should be free *i. e.* in β-position. The IR spectrum of the compound exhibits bands at 1660 cm^{-1} and 1626 cm^{-1}characteristic of unco-ordinate carbonyl group and a carbonyl group co-ordinated with α-hydroxy group respectively in an anthraquinone. Thus one of the two hydroxy groups should be present in α-position while the other one in β-position. In all, there may be twelve structures having these groups with different possible orientation in the anthraquinone nucleus. But nine of these are already known in the literature[11,12,13]. Thus the tora colouring matter C can have any one of the following structures.



(a)



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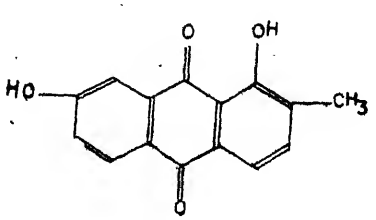

(c)

Tora Colouring Matter C

EXPERIMENTAL

10Kg of the crushed seeds were extracted with petroleum ether (40-60°). The solvent was distilled off and last traces were evaporated over water-bath. The oil was left over night when a dirty brown product settles down, which was filtered and washed well with petroleum ether to remove the traces of adhered oil to the colouring matters. On leaving the oil for a month or so, a fresh crop of colouring matters settles down.

Isolation and Purification of Colouring Matters :

The crude brown product so obtained was dissolved in dry acetone and impurities were filtered off. The filtrate was evaporated to dryness and the residue was refluxed with chloroform and on cooling a shining orange-red crystalline compound (A) was deposited. The product was filtered out and recrystallised from hot chloroform to a constant m. p. 236-38°. The mother liquor was concentrated to one third of its volume and on cooling, a dark red product began to separate. This product was filtered out and on recrystallisation gave a dark red compound B, m. p. 196-98°. The filtrate so obtained was evaporated to dryness and the residue on recrystallisation from benzene gave a yellow compound C., m. p. 229-30°C. Thus the crude product was seprated into three definite components isolated from cassia tora.

The crude colouring matter was also confirmed to be a mixture of three hydroxy anthraquinone type compounds by paper chromatography[9] and individual colouring matters were found pure and single entity.

Properties and Reactions of Tora Colouring Matter A :

The tora colouring matter A is an orange red crystalline compound, m. p. 236-38°C soluble in acetone, benzene, ethanol and hot chloroform. It gave dirty green colouration with ethanolic ferric chloride. A yellow solution was produced when dissolved in alkali which was decolourised by the addition of mineral acids. It gives yellow orange-colour with methanolic magnesium acetate (found C=66·0%, H=3·4%, OMe=11·1%, M. W. (Rast) 280; $C_{15}H_{10}O_5$ requires C=66·6%, H=3·7% OMe=11·48% (for one methoxyl group) M. W. 270.

Diacetate :

100 mg of Tora colouring matter A was taken in conical pyrex flask, to it 2-2·5 ml of acetic anhydride and 0·4 gm of fused sodium acetate were added. The mixture was refluxed over a sand bath for eight hours. The contents were cooled and poured in iced water and the product so obtained was recrystallised from acetone while a pale green acetylated product m. p. 219-21°C was obtained.

(Found Acetyl group =23·5%; $C_{15}H_8O_3(O\,COCH_3)_2$ requires 24·2%)

Methyl ether :

50 mg of the colouring matter A was taken in 50 ml pyrex flask with 2 ml dimethyl sulphate (in excess) and 0·5 gm of anhydrous potassium carbonate. The mixture was refluxed over a water bath for 20 hours. The solvent was evaporated when a brown precipitate settled down. It was filtered and washed well with water and dried in vacuum desiccator. It was recrystallised from methanol, when a brown crystalline compound m. p. 215° (decomp.) was obtained.

(found methoxyl group=31·2%; $C_{14}H_5O_2(OCH_3)_3$ requires = 31·26%).

Reaction with Potassium permanganate :

300 mg of tora colouring matter A was dissolved in 10 ml of 5% sodium hydroxide solution and 2·5% solution of potassium permanganate was added till the pink colour persisted. It was heated over a water-bath and the excess of potassium permanganate was destroyed by few drops of sodium bisulphite solution. The black precipitate of manganese dioxide formed, was dissolved by adding a little concentrated hydrochloric acid, when a white precipitate appeared. It was filtered and washed well with water, recrystallised from ethanol, m. p. 208-10°C. The compound was found an acid and gave fluorescein test. It was confirmed to be phthalic acid by mixed m. p. No other product could be isolated from this reaction.

Zinc-dust distillation :

Arintimate mixture of 500 mg of tora colouring matter A and 3 g. of zinc dust were heated in an atmosphere of nitrogen at about 400°C. Very light yellowish vapours rising from the flask were dissolved in benzene. The benzene solution was concentrated to one third, when a small quantity of yellow crystalline compound m. p. 214-16°C was obtained. This gave yellow picrate m. p. 135-137°. This was inferred to be anthracene which was confirmed by finding out mixed m. p. with authentic sample of anthracene.

Tora Colouring Matter B :

The tora colouring matter B is a dark red crystalline substance, soluble in acetone chloroform, carbon tetra chloride and ethanol. It gave blue-green colour with ethanolic ferric chloride and red colour with alkali, which undergoes a sharp change by the addition of dilute mineral acids. I gave yellow-orange colour with methanolic magnesium acetate.

Found C=67·9%, H=4·1%, OMe=11·0%, M. W. (Rast)=292; $C_{16}H_{12}O_5$ requires C=67·6%, H=4·2, OMe=10·9% (for one OMe) M. W. 284).

Diacetate :

Acetylation of tora colouring matter B was carried out in the same way as that of tora colouring matter A. The acetyl product was recrystallised from acetone, when shining flakes m. p. 220-222 C were obtained (found Acetyl group=23·0%, $C_{16}H_{10}O_3$ $(O.COCH_3)_2$ requires 23.3%).

Methyl ether :

Methylation of tora colouring matter B was carried out as in the case of tora colouring matter A. The methylated product was recrystallised from methanol when a brown crystalline compound m. p. 174° (decomp.) was obtained. Found OMe=28·9%; $C_{15}H_7O_2$ $(OCH_3)_3$ requires=29·8%).

Zinc-dust distillation :

Zinc dust distillation of tora colouring matter B was done in a similar manner as in the case of tora colouring matter A, In this case a yellowish compound m. p. 202-03°C was obtained, which was inferred to be 2-methylanthracene and it was identified by finding out the mixed melting point. When the compound was distilled in the presence of alkali and zinc-dust, a yellow compound was obtained m. p. 174°-75°C, which was identified to be 2-methyl anthra-

quinone by finding, the mixed melting point with authentic sample of 2-methyl-anthraquinone.

Tora colouring matter C :

The tora colouring matter C, is a yellow crystalline compound m. p. 229-30°C soluble in acetone, benzene, chloroform, carbon tetra chloride and ethanol. It gave blue-green colour with ethanolic ferric chloride, when dissolved in alkali, an intense-red coloured solution was obtained, which undergoes a sharp indicator type change by the addition of mineral acids. It gave a pink colour with methanolic magnesium acetate.

(found C = 71·0%, H = 3·8%, M. W. (Rast) 264; $C_{15}H_{10}O_4$ requires C = 70·86%, H = 3·9%, M. W. 254).

Diacetate :

It was acetylated in the manner as other two colouring matters A and B have been done. The acetylated product was recrystallised from acetone, when a brown compound m. p. 268-7°C (decomp.) was obtained.

(found Acetyl group = 25·00%; $C_{15}H_8O_2$ $(OCOCH_3)_2$ requires 25.44%).

Methyl ether :

Methylation of the tora colouring matter C was done in the same way as that of tora colouring matter A. The product was ecrystallised from methanol when a red crystalline compound m. p. 210° (decomp.) was obtained.

(found Methoxyl group = 21·4%, $C_{15}H_8O_2$ $(OCH_3)_2$ requires = 21·9%).

Zinc-dust distillation :

The zinc dust distillation of the tora colouring matter C was carried out similarly as in the case of colouring matter B. The products obtained were identical as in the case of colouring matter B.

ACKNOWLEDGMENT

One of the authors (R. K. S.) is greatful to C. S. I. R., New Delhi for the award of Junior Research Fellowship.

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CHEMICAL EXAMINATION OF *LITSEA CONSIMILIS*, NEES—STUDY OF ALKALOIDS

By

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Chemical Laboratories, Th. D. S. B. Government College, Naini Tal

ABSTRACT

An alkaloid Laurotetanine $C_{19}H_{21}O_4N.H_2O$, m. p. 125°, has been isolated from the bark and the seeds of *Litsea consimilis*. A hydrocarbon m. p. 214°, probably a triterpenoid has been also isolated from the seeds of the plant.

Litsea consimilis, Nees, (N. O. Lauraceae, local name Chirara, Bailara) is a shrub found abundantly in Kumaon region. The extract of the seeds is locally used for the treatment of oedema.

No work is reported in any standard journal about the alkaloid contents of the plant.

According to present studies one alkaloid *Laurotetanine* $C_{19}H_{21}O_4N.H_2O$ m. p. 125° has been isolated in pure from by two different methods from the bark and seeds of *L. consimilis*.

Laurotetanine has been isolated by Greshoff,[1] Fillipo[2], Gorter[3], Spath[4] and Barger[5] from *L. chrysocoma*, *L. citrate* and *Litsea cubeba*.

The amount of alkaloid present in the bark was found to be changing with the change of season. The alkaloid contents were found to be maximum in spring (in the bark) and minimum in the month of November. The seasonal change in the alkaloid content is probably due to movement of the alkaloid from the bark to the seeds, after setting in of fruits in the shrub.

About 0.1% yield of pura Laurotetanine alkaloid was obtained by alcoholic extraction method.

The concentrated chloroform extract of the defatted seeds was chromatographed over neutral alumina and eluted with petroleum ether, benzene, acetone, methyl alcohol, chloroform and their mixtures in different proportions. As such an alkaloid Laurotetanine m. p. 125° and needles of an organic hydrocarbon m. p. 214° (probably a triterpenoid) were obtained.

The alkaloid was identified by preparing various salts and derivatives such as hydrochloride, oxalate, picrate, dibenzoyl and thiocarbamide and also by the characterisation of the oxidation product.

EXPEREMENTAL

Isolation. 1 kg. of dried bark was soaked in about 2 litres of rectified spirit, in a 5 litre pressure pyrex flask and kept over night. Next day the contents were boiled under reflux condenser for about an hour and filtered hot. The alcohol was completely distilled off from the extract uuder reduced pressure.

The dried tarry mass was dissolved in about 80 ml. of hot glacial acetic acid. This solution was poured into 400 ml. of distilled water and stirred vigorously for about half an hour by an electric stirrer, and filtered. To the

filtrate, ammonia was added drop by drop till the solution remained only faintly acidic to litmus to give a light brown precipitate 'A'. To the filtrate from 'A' excess of ammonia was added till the contents were just alkaline to give a pale yellow voluminous precipitate 'B'.

Precipitate 'A' was redissolved in glacial acetic acid and the above process repeated to get more quantity of 'B'.

Residue 'B' was dissolved in chloroform, and to this solution 20% (v/v) acetic acid was added and contents shaken thoroughly. The acid layer (collected separately) was made just alkaline by ammonia, to get back the base 'B'. After filtration the residue was washed with water and dried in a vacuum desiccator. The dried light brown mass was thoroughly mixed with powdered sand (acid treated, B. D. H. quality) and extracted with ether in a soxhlet. A crystalline mass was collected after cooling; from the flask containing the ether solution. The crystalline mass was dissolved in acetone followed by an addition of a drop of water. After few hours, almost colourless needles of an alkaloid m. p. 125° were collected. It did not darken on exposure to light.

Found C=66.6%, H=6.5%, O=22.4% & N=4.5%

while for $C_{19}H_{21}NO_4$. H_2O ; C=66.08%, H=6.67%,
O=23.18% and N=4.06%.

Hydrochloride of the base. The hydrochloride of the base was prepared by passing dry HCl into an alcoholic solution of the alkaloid. The alcohol was evaporated off slowly to get white needles of the hydrochloride m. p. 245°.

found chlorine=9.59%

$C_{19}H_{21}O_4N.HCl$ requires=9.76%

Oxalate of the base. Equal amounts of oxalic acid and the alkaloid were mixed in alcoholic solutions, warmed and stirred. After concentrating the solution a crystalline salt settled down, filtered and dissolved the residue in hot alcohol to get crystalls of oxalate m. p. 233° after cooling.

Picrate of the base. To the hot aqueous solution of the hydrochloride of the base, saturated aqueous solution of picric acid was added. A light yellbw powder came down after stirring the contents with a glass rod. The precipitate was dissolved in alcohol to get yellow needles of picrate of the base m. p. 147°; on slowly evaporating off the alcohol.

Dibenzoyl derivative. About 1 g. of the alkaloid was taken and dissolved in about 10 ml. of 5% KOH, then about 2 ml. of benzoyl chloride was added. Corked the flask and shaken vigorously for about 15 minutes. A colourless solid which appeared after some time was cellected and crystallised from acetone to get the benzoate m. p. 169°.

Thiocarbamide. In a 100 ml. flask (R. B.) 2 g. of alkaloid, 3 ml of CS_2 and 5 ml of absolute alcohol were taken and the contents were refluxed for about 10 hours. After removing excess of CS_2, dilute HCl was added to the residue and contents shaken thoroughly and filtered to remove any unreacted alkaloid. The residue was dried in a vacuum desiccator and then crystallised from boiling alcohol to give the thiocarbamide m. p. 154°.

Alkaline $KMnO_4$ oxidation. 2 g of the base, 0.2 g of KOH and 25ml of 10% $KMnO_4$ solution were taken in a 250 ml R. B. Flask and refluxed for about an hour. Concentrated HCl was then added to neutralise excess of $KMnO_4$. The clear solution thus formed was evaporated to dryness, on a boiling water bath and then further dried in a vacuum desiccator. To the dry mass hot alcohol was added and filtered. After evaporating off alcohol from the filtrate, colourless needles m. p. 165° were obtained.

The compound was identified as 1 : 2 dimethoxy benzene 3 : 4 : 5 tricarboxylic acid m. p. 165°.

The base was thus identified as Laurotetanine $C_{19}H_{21}NO_4.H_2O$ m. p. 125°.

Column chromatography. The concentrated chloroform extract was chromatographed over alumina and eluted with petroleum ether (40-60), petroleum ether (60-80), benzene, acetone, chloroform and methyl alcohol and their mixtures in different proportions and the different eluates were examined.

As such two organic compounds were isolated.

Compound no. 1. The benzene-chloroform fraction gave a product m. p. 214° which after further crystallisation from hot ethanol gave colourless needles m. p. 214°. The compound gave no test for the presence of N, S and halogens and did not burn with smoky flame.

The compound responded to some tests for a triterpenoid.

Compound no. 2. The chloroform methyl alcohol fraction gave a product which was crystallised from acetone (with a drop of water) to give needless of m. p. 125°. The compound was found to be the same alkaloid i. e. Laurotetanine.

ACKNOWLEDGEMENT

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CHEMICAL EXAMINATION OF FRAXINUS FLORIBUNDA— STUDY OF GLYCOSIDES

By

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ABSTRACT

Three glycosides, Cichoriin m. p. 210°, Fraxin m. p. 200-205° and Aesculin m. p. 200-205° have been isolated from the bark of Fraxinus Floribunda, for which no analytical data is found in any standard journal. A good yield of mannitol (about 1%) m. p. 167° was also obtained from the same.

Fraxinus floribunda:—(N. O. Oleaceae) is a large tree found abundantly in the Kumaon region. The bark of the tree is reputed for its medicinal use as a tonic febrifuge[1].

The present investigation deals with the examination of the bark of the tree (from Kumaon region) for its glycoside content.

The bark when immersed in water make the extract highly fluorescent due to the presence of glycosides.

Though Aesculin and Fraxin have been isolated by various authors from the bark of some Fraxinus species, cichoriin has not been so far reported to be present in the bark of any Fraxinus tree. However very recently Steinggen[3] reported the presence of cichoriin in the blossom of Fraxinus ornus.

Three glycosides with Rf values 0·51, 0·55 and 0·59 were detected by papyrographic method using *n*-butanol acetic acid-water (4:1:5) system. The aglucone with Rf value 0·82 was identified to be aesculetin.

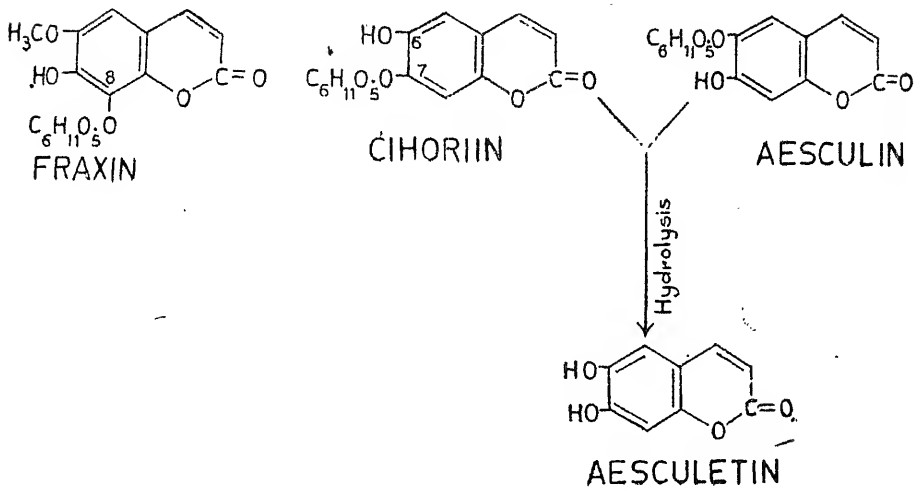
Methods applied by various authors[4,5] to isolate Aesculin and Fraxin did not succeed for the isolation of glycosides from the bark of Fraxinus floribunda. Separation of the three glycosides was found possible only by the use of column choromatography when a concentrated solution of the plant extract was choromatographed over chromatographic cellulose and then eluted by benzene, ether, acetone, methyl alcohol and their mixtures. The column after complete development of zones (zones observed under U. V. light) was cut into differently coloured fluorescent zones and collected separately. As such three glycosides were isolated i. e. Fraxin, Aesculin and Cichoriin.

The identity of cichoriin and Aesculin was established by the study of their mixed melting points with the authentic samples, Rf values and the study of their properties. Both cichoriin and Aesculin on hydrolysis gave an aglucone aesculetin $C_9H_6O_4$ m. p. 265—70°. The aglucone was identified by the study of its derivatives, oxidation product, abs-spectra, mixed melting point and Rf value determination.

The amount of Fraxin obtained was small and it was identified by its Rf value and melting point only as reported in the literature.

Aesculetin was also directly obtained by boiling the residue of the alcoholic extract with dil H_2SO_4.

The chart below shows the relationship between compounds mentioned above.



EXPERIMENTAL

1 Kg. of the freshly collected crushed bark of Fraxinus floribunda was taken in a 5 litre flask along with about 3 litres of 95% ethanol and kept over night. Next day the filtered percolate thus obtained was allowed to stand in a breaker for some time when a white crystalline precipitate 'A' separated out. On concentrating the solution a white crystalline precipitate 'B' separated out, which was further collected on distilling off about 3/4 of the alcohol. After removing alcohol completely by distilling under reduced pressure, the dry residue was thoroughly washed with cold water to get an aqueous extract of the residue. From this extract further quantities of colourless crystalline mass 'A' was again collected after evaporating the cold aqueous extract to dryness. The dry residue was then treated with boiling water and filtered hot. The filtrate on cooling gave almost a colourless mass 'B'.

The insoluble residue was refluxed with alcohol to get pure greenish yellow crystals of a compound 'C' m. p. 265—70°.

Residue "A":—The residue "A" was crystallised from alcohol to give fine colourless needles of mannitol m. p. 167° identified by determining the mixed melting point with authentic sample and also by preparing the hexa-acetate derivative m. p. 119°.

Residue "B":—The residue "B" obtained from different fractions was collected and dissolved in hot alcohol in an attempt to crystallise the glycoside. But being highly hygroscopic and with some impurities of other glycosides, it could not be crystallised. However the m. p. was determined and found to be 185°. As the residue "B" reduced Fehlings solution only after hydrolysis with strong HCl it was considered to be a glycoside.

Residue "C" :—The compound "C" was refluxed with alcohol and then the solution boiled with a pinch of animal charcoal and filtered hot. On cooling greenish-pale yellow needles of the aglucone m. p. 265—70° separated out.

On combustion the percentage of carbon, hydrogen, and oxygen was found to be 60·34°/₀, 3·40°/₀, and 36·26°/₀ respectively. The molecular weight (Rast's method) was found to be 182. Therefore the formula of the compound is $C_9H_6O_4$ (m. w. 178) which requires C—60·67°/₀, H=3·37 and oxygen 35·96°/₀.

The compound was found having a coumarin nucleus because it responded to Feigel's[2] spot test for coumarins, when it formed a violet fluorescent solution on boiling with resorcinol in 80°/₀ sulphuric acid and then rendered alkalines and also gave Mg-HCl colour test.

The number of OH groups was found to be two (phenolic) and therefore the compound is a dihydroxy coumarin.

IDENTIFICATION OF GLYCOSIDES IN RESIDUE "B" BY PAPYROGRAPHIC METHOD.

As the glycoside "B" could not be crystallised its identity was revealed by papyrographic method, when cichoriin was found to be the main constituent with some impurities of Aesculin, Aesculetin and Fraxin.

Procedure:— About 0·01 g of the crude glycoside (fraction "B") was dissolved in hot alcohol and spotted on Whatman no. 1 filter paper using *n*-butanol-acetic acid-water (4:1:5) system. Simultaneously cichoriin and aesculetin (supplied by Dr. E. Steingger of Bern University) were spotted on the same paper. The residue "C" was also spotted. The results are tabled below :

Sl. No.	Name of the organic compound	Fluorescence in U. V. light	Colour after spraying with KOH in visible light	Fluorescence in U. V. light after KOH spraying	Rf value
1.	Cichoriin	flesh coloured	Yellow brown	Yellow	0·55
2.	Aesculetin	blue green	light green	Yellow green	0·82
3.	Glycosides "B"	(a) blue green	light green	Yellow green	0·82
		(b) strong blue	light brown	strong blue	0·59
		(c) flesh coloured	yellow brown	Yellow	0·55
		(d) green coloured	pale yellow	Green	0·51
4.	Aglucone "C"	blue green	light green	Yellow green	0·82

Thus the presence of cichoriin in the glycoside "B" was confirmed. Presence of small amounts of aesculetin (Rf value 0·82), aesculin (Rf value 0·59) and Fraxin (Rf value 0·51) was also confirmed in "B". The aglucone "C" was identified as aesculetin (Rf value 0·82).

SEPARATION AND IDENTIFICATION OF COMPOUNDS FROM RESIDUE "B" BY COLUMN CHROMATCGRAPHY

Alcohol was completely removed from the alcoholic extract of the bark and from the dried mass mannitol was separated out and then the hot aqueous extract was taken and evaporated to dryness on boiling water bath. Alcohol was added to the residue and the concentrated extract was chromatographed over chromatographic cellulose, eluted with benzene, ether, acetone and methyl alcohol and their mixtures in different proportions. After complete development, the column was put under U. V. light when different coloured zones were observed. The three fluorescent zones : the green, flesh coloured and blue zones were separated by means of a nickel spoon, the process being carried out under U. V. light.

The three zones collected separately were soxhleted for few hours using alcohol as solvent. The glycosides were thus collected after removing alcohol and further crystallised from rectified spirit to give three different glycosides m. p. 200–205°, 210° and 200–205° from green flesh coloured and blue fluorescent zones respectively and identified as fraxin, cichoriin and aesculin respectively as described below.

Fraxin:—Out of the three fluorescent zones the upper most zone gave fraxin m. p. 200–205°. The green fluorescent spot in papyrographic identification was also fraxin. Fraxin melts a 200 - 205° and is reported to have the same Rf value (0·51) with similar coloured fluorescent spot.

Cichoriin:—Out of the three zones, the central zone (flesh coloured under U. V. light) gave the glycoside cichoriin m. p. 210°. There was no depression in m. p. when it was mixed with an authentic sample.

The glycoside was found to be non-fluorescent in aqueous or in alcoholic solution. On hydrolysis the glycoside gave an aglucone identified as aesculetin m. p. 265–70°.

Aesculin:—The lower most dark blue florescent zone gave the glycoside aesculin m. p. 200 - 205° after soxhleting the zone with alcohol. There was no depression in m. p. when mixed with an authentic sample of aesculin. The glycoside gave a fluorescent (blue) solution in alcohol or water. When boiled with mineral acids the glycoside gave the same aglucone i. e. aesculetin m. p. 265–70°.

Hydrolysis of cichoriin and Aesculin:— 0·5 gm. of each cichoriin and aesculin was dissolved in hot distilled water (25 m. l.) separately to which dilute sulphuric acid (10 ml. of 4N) was added and the contents boiled for about 2 hours and kept as such over night. Next day needles of the aglucone were collected. The aglucone was dissolved in hot methanol and boiled with a pinch of animal charcoal and filtered, greenish yellow needles of the aglucone m. p. 265–70° were thus collected.

Identification of the aglucone:—The aglucone was found to be a dihydroxy coumarin and was identical with residue "C" described above. Various derivatives such as diacetyl m. p. 130–31°, dibenzoyal m. p. 185° and dimethyl m. p. 142° were prepared by the usual methods and found to be identical with the similar derivatives of aesculetin (6 : 7 dihydroxy coumarin).

Alkaline $KMnO_4$ oxidation:—The aglucone (0·1 g) was boiled with alkaline $KMnO_4$ (15 ml. 10%) for about an hour. Excess $KMnO_4$ was neutralised by concentrated HCl. The whole solution was evoporated to dryness on boiling water

bath. To the dry residue hot alcohol was added and filtered. On evaporating off alcohol slowly needle of an organic acid m. p. 217° separated out. The acid was identified as 2: 4: 5 trihydroxy benzoic acid.

Absorption spectra:—The absorption spectra of the recrystallised aglucone was taken with help of Hilger U. V. Spectrometer. A dilute solution of the compound in ethanol (95$_0$°/$_0$ was taken and the concentration adjusted by measuring the optical density of the solution. The absorption curve thus obtained showed absorption peaks at 3500A°, 2550A° and 2300A°.

Sugar residue:—The glycoside 'B' was boiled with HCl for two hours, cooled and filtered. The filtrate was evaporated to dryness and the aqueous extract of the residue was chromatographed along with known sugars using *n*-butanol-acetic acid-water (4:1:5) system on paper when glucose with Rf value 0·19 was detected. Thus the sugar concerned is glucose.

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CHEMICAL EXAMINATION OF *Terminalia paniculata* ROTH.

By

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SUMMARY

Analyses of *Terminalia paniculata* Bark shows that it is made up of 27 per cent of extractives that can be removed by successive extraction with petroleum ether, chloroform alcohol and water, 28 per cent of carbohydrate, 33 per cent of 'lignin' and 9.6 per cent of ash. The bark does not contain any alkaloids. It contains 14 per cent of tannins, which have a pyrogallol nucleus in the molecule rather than a catechol nucleus along with gallic acid. β-sitosterol has been isolated and identified from the petroleum ether extract of the bark.

Terminalia paniculata Roth. is a very large tree occuring in the deciduous forests of southern India. It yields a grey wood, with very hard darker heartwood. which is a splendid substitute for teak.[1] Its bark 0.6 cm thick, dark brown, peeling off in flat flakes contains tannins and is said to be used for tanning purposes.[2] The bark also possesses cardiotonic and diuretic properties.[3] Although chemical examination of some other species of *Terminalia*[4-7] has been reported, work on this species was yet to be undertaken. The present paper deals with the chemical examination of *Terminalia paniculata* bark.

EXPERIMENTAL

Sources and preparation of the raw material:—The chemical studies were conducted on the authenticated bark sample, kindly supplied by the Range Officer, Tunacadavu Range, Madras State. The pieces of the bark were first reduced in size and then pulverized to 20 mesh size. The ground material was stored in tight containers to avoid contamination and possible deterioration.

Extraction studies:—Suitable, accurately weighed samples of powdered material were extracted successively with a series of solvents in a Soxhlet continuous extraction apparatus. The solvents were used in the order of increasing polarity, and the extraction with a given solvent was continued until an aliquot of the colourless percolate left no residue when evaporated to dryness. The percentages of sequential extractives are recorded in Table I.

TABLE I.

Percentages of sequential extractives of the bark.

Sl. No.	Solvent	Percentage of extractives*
1.	Petroleum ether (60-80°)	0.27
2.	Chloroform	0.36
3.	Ethanol	9.72
4.	Hot water	16.58
5.	1-per cent Sodium hydroxide	33.31

*Percentages are expressed on the oven-dried weight. of the bark.

Proximate analyses :—These were made using methods generally applicable to wood. The results of analyses of the whole bark, bark extracted with petroleum ether, chloroform, ethyl alcohol, and water, and bark subsequently extracted with hot 1-per cent aqueous sodium hydroxide solution are given in Table II.

TABLE II.

Proximate chemical analyses of the original and extracted barks.

Sl. No.		*Terminalia paniculata* bark	Neutral-solvent extracted bark*	Neutral-solvent and sodium hydroxide extracted bark
1.	%Ash	9.63	7.77	8.84
2.	%Lignin	32.76	43.69	41.23
3.	%Sugars**	31.18	47.66	49.17
4.	%Pentosans in the reducing sugars	43.08	30.38	28.50

Percentages are expressed on the oven-dried weight of the material.

* Material extracted by petroleum ether, chloroform, ethyl alcohol, and water.

** Reducing sugars calculated as glucose.

Alkaloids :—The chloroform extract of the bark (100 g) was examined for alkaloids. Negative results were obtained with Mayer's reagent, Wagner's reagent, Fröhde's reagent and picric acid T. S., conclusively indicating the absence of alkaloidal substance in the bark material.

Tannins :—The tannin content of the bark was determined according to the 1955 Official Methods of Analysis of the Association of Official Agricultural Chemists.[8] The results are reported on the oven-dried weight of the bark.

	Per cent
Tannin	13.92
Non-tannin	4.21

An attempt was made to classify the tannins according to their specific chemical reactions with various reagents. In order to prevent one intense colour from obscuring or masking another, several tannin solutions of varying concentrations were prepared. Positive results were obtained with ferric chloride T. S. and ammonium molybdate, while negative results were obtained with fresh bromine water and vanillin-hydrochloride.

The appearance of a blue black colour with ferric chloride T. S. indicated a pyrogallol nucleus (three adjacent free phenolic groups) in the molecule, rather than a catechol nucleus (two adjacent free phenolic groups). Positive test with ammonium molybdate indicated the presence of gallic acid.

Examination of Petroleum Ether Fraction : Continuous extraction of the powdered bark (2 kg) with boiling light petroleum (b. p. 60-80°) for 18 hours afforded a pale yellow solution containing suspended gelatinous solid in small quantities, which was collected and is being identified. The orange red oil (3.43 g, 0.2%) obtained by evaporation of the clear petroleum solution was divided into acidic and neutral fractions by shaking with 0.5 *N* sodium hydroxide. The neutral fraction which constituted about 85 per cent of the oil was chromatographed over Brockmann alumina in benzene. Benzene as an eluant isolated a colourless crystalline material, which crystallized from methanol as plates, m. p. 138-39° ; $[\alpha]_D$ - 34° (chloroform). It gave bluish pink coloration with Liebermann-Burchard reagent and a positive Salkowski reaction. The acetate prepared with pyridine and acetic anydride melted at 127-28° and the benzoate prepared with pyridine and benzoyl chloride melted at 143° ; $[\alpha]_D$ −13° (chloroform). The physical data of all these compounds resembled fairly with those of β-sitosterol and its corresponding derivatives and a mixed melting point of the benzoate with an authentic sample of β-sitosterol benzoate remained undepressed. So the petroleum ether extract was found to contain β-sitosterol.

Further work is in progress on the examination of the other extracts of the bark.

ACKNOWLEDGEMENT

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SAPONINS AND SAPOGENINS—PART XX

CHEMICAL EXAMINATION OF PITHECOLOBIUM DULCEBENTH (INGA DULCIS WILLD)

By

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SUMMARY

The seeds of Pithecolobium dulce Benth, obtained from Kerala by defatting and alcohol extraction give a mixture of saponins, which on sulphuric acid hydrolysis gives the sapogenins. On acetylation and crystallisation it gave a mixture of two acetates, one of which has been identified as proceric acid; acetate m. p. 286-88; acetate methyl ester m. p. 269-74 (Cf. Proceric acid acetate m. p. 288-90; proceric acid acetate methyl ester m. p. 268-70 and an unidentified acid genin; acetate m. p. 215-220; acetate methyl easter m. p. 217-22.

Pithecolobium dulce Benth, a member of the family Leguminoseae sub-family mimoseae is a plant common in South India. A review to the literature showed that no work on the saponin contents of this plant has been done. Being engaged in the systematic study of saponins and sapogenins from the family leguminoseae, it seemed of interest to study the seeds for their saponin and sapogenin contents. While this work reached completion a paper on the study of the seeds of this plant by Mitra et al[1] appeared reporting the isolation of a saponin m. p. 175-81, sapogenin, pithogenin ($C_{28}H_{44}O_4$) m. p. 207-8 $[\alpha]_D$ +81°, a sterol glycoside B m. p., 278-80, aglycone m. p. 259–60 and a flavone m. p. 209-306 from the seeds and a sterol glycoside m. p. 282-86; aglycone 136-38 and certain amino acids from the mesocarp, but our results are not in agreement with this work. The seeds of Pithecolobium dulce obtained from M/S Johnson Sons & Co., Allepey were defatted and then exhausted with alcohol. The recovery of the solvent left a gummy mass which was successively extracted with ether, petroleum ether, carbon-tetrachloride and acetone. The residue was then dissolved in alcohol and the saponin precipitated by addition to a large volume of ether. The saponin thus obtained was dissolved in a large volume of water and hydrolysed with sulphuric acid (10%) by heating on a water bath followed by refluxing. The genin which separated was filtered and washed free of acid. It was then purified by refluxing with alcoholic solution of sodium hydroxide and extraction of the aqueous solution with ether. Evaporation of the ethereal layer gave a neutral fraction. The alkaline solution was acidified with hydrochloric acid and the precipitated acid genin acetylated by pyridine and acetic anhydride in the cold. The acetate on repeated crystallisation from methanol gave two products A m. p. 286-88 and B m. p. 215-22°.

The acetates were methylated with diazomethane. The two methyl esters from the acetates A and B had melting points 269-74 and 217-22 respectively.

The acetate m. p. 286-88; acetate methyl ester m. p. 269-74 has been identified as Proceric acid,[2] earlier isolated from the seeds of Albizzia procera Benth from Maharashtra, by mixed m. p. of the acetate methyl ester with an authentic sample of acetate methyl procerate and superimposable infra-red spectra.

EXPERIMENTAL

All the melting points recorded in this paper have been taken on Kofler's hot microscopical stage and are corrected. The infra-red spectra were recorded in Nujol mull on Infracord Model 137.

Defatting. Well powdered seeds (960 gms) were extracted with light petroleum ether (40-60) thrice. The recovery of the solvent left the oil (89 gms.). The exhausted seeds were dried before further operation.

Extraction. The defatted seeds were extracted with alcohol (3 l.) 4 times and the combined extracts evaporated to yield a gummy mass. It was successively extracted with ether, petroleum ether, carbon tetrachloride and acetone. The residue was dissolved in the minimum quantity of alcohol and precipitated by addition to a large volume of ether (3·5 l.).

Hydrolysis of the saponin. The saponin was dissolved in water (3·5 l) and hydrolysed with sulphuric acid (350 gm) by heating the solution first on a water bath for an hour followed by refluxing the solution for another hour. The sapogenin formed was filtered and washed free of acid and dried (7·8 gms.).

Separation of acid and neutral genins.

The genin was dissolved in alcohol (250 ml) containing sodium hydroxide (25 gms) and refluxed for 1/2 hours and then the volume concentrated to about half. The solution was then poured into water and extracted with ether. Recovery of the ether solution yielded the neutral portion.

The aqueous layer was acidified with hydrochloric acid and the precipitated genin filtered, washed free of acid and dried (6·25 gms).

Acetylation: The acid genin was dissolved in pyridine (25 ml) and acetic anhydride (25 ml) added. The mixture was poured into crushed ice, solid obtained filtered, washed free of acid and pyridine, dried, taken in methanol and charcoaled. On repeated crystallisation from methanol it separated into two fractions A m. p. 286-83 (Cf. proceric acid acetate m. p. 288-90) and B. 215-20.

Methylation of A.

The product was dissolved in ether and an ethereal solution of diazomethane added to it. The mixture was kept overnight, ether evaporated and residue crystallised from methanol as needles m. p. 269-74 (Cf. acetate methyl ester of proceric acid m. p. 268-70) Mixed m. p. with methyl procerate acetate 270-74.

Methylation of B.

The methyl ester of B was prepared in the above manner and crystallised from methanol m. p. 217-22.

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SAPONINS AND SAPOGENINS XIX - THE STUDY OF THE SAPONIN FROM THE SEEDS OF ALBIZZIA STIPULATA BOIV.

By

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SUMMARY

The seeds of Albizzia stipulata from Maharashtra yield a colourless saponin, which on sulphuric acid hydrolysis gives a genin m. p. 270-71°, acetate m. p. 231-32°. It has been identified as Acacic acid by mixed melting point and Infrared spectra.

In the course of our systematic study on the sapogenins from the various species of Albizzia, it has been noted that the seeds of the members of family leguminoseae specially Albizzia; studied so far, contain, triterpenic saponins and sapogenins belonging to the β-amyrin group varying from plant to plant. [1-6]

No mention of the work on the saponins and sapogenins of the seeds of Albizzia stipulata is found in the literature. The seeds of Albizzia stipulata obtained from Maharashtra, on alcoholic extraction and usual subsequent treatment gave a saponin, which on sulphuric acid hydrolysis has been found to give an acid genin; m. p. 270-71° giving on acetylation an acetyl lactone m. p. 231-32°. The genin has been identified as Acacic acid* by mixed melting point and infra-red spectra with authentic samples of acacic acid. (Cf. Acacic acid m. p. 280-81° and acetyl lactone m. p. 235-36°) [7-8]. On selenium dioxide oxidation the acetyl lactone gives a compound m. p. 210-12° showing λ_{max}^{EtOH} 255 mμ and 265 mμ.

The genin on treatment with diazomethane gives a methyl ester m. p. 260-62°. The chromic acid oxidation of the methyl ester gives a nor diketo methyl ester m. p. 338-42°.

It may be mentioned here that acacic acid has earlier been isolated from the bark of Acacia intsia Willd[7,8] and later on from the seeds of Acacia intsia and Acacia concinna.[9] It has also been reported in the bark of Albizzia lebbek Benth.

Further work on the constitution is in progress.

EXPERIMENTAL

All the melting points recorded in this paper have been taken on Koflers Hot Microscopical stage and are corrected. The infra-red spectra were taken on Perkin Elmer Spectrometer Model 137 (Infracord) by the author in these laboratories and through the courtsey of Prof. R. Tschesche Bonn, Germany, on the Perkin Elmer Spectrometer Model 221. U. V. Spectra has been taken on Beckman Spectrophotometer model DU in these laboratories.

Extraction of the saponin : The well defatted seed powder (200 gm) was exhausted with ethyl alcohol in a soxhlet apparatus. The alcohol, was recovered and the brown syrup left over was successively treated with petroleum ether, ether, carbon tetrachloride, chloroform and acetone to remove all impurities soluble in these solvents. The product was dissolved in alcohol and precipitated a number of

*Z. Naim Ph. D. Thesis, Aligarh Muslim University, 1961, thought the genin of this plant to be a new one.

times by adding the solution to a large volume of ether/acetone. The colourless powder thus obtained gave all the tests for saponins.

Hydrolysis of the saponin : The saponin (1·0 gm.) was dissolved in water (1 litre) and heated with sulphuric acid (10%) on a waterbath for an hour when a colourless solid separated. It was refluxed for another hour on a heating mantle to ensure complete hydrolysis. The genin was filtered and washed free of acid.

Separation of the acid and the neutral genins : The genin (250 mg.) was refluxed with methyl alcoholic potassium hydroxide (10%) on a waterbath for one hour. Half of the methyl alcohol was recovered and the reaction mixture poured in water. It was extracted with ether a number of times, all the ethereal layers were combined and washed free of alkali. Recovery of the ether did not leave any neutral genin. The alkaline solution left over after the ether extraction was acidified with hydrochloric acid when a colourless precipitate of the acid genin was obtained. It was filtered and washed free of hydrochloric acid.

Acetylation of the acid genin : The acid genin (100 mg) was dissolved in pyridine (25 cc) and heated with acetic anhydride (30 cc) on a water bath for two hours. The acetate was filtered, washed a number of times with water to remove pyridine, dried and crystallised from methyl alcohol as fine colourless needles m. p. 231-32° (Cf. acetyl acacic acid m. p. 235-36°). It did not depress the melting point when mixed melting point was taken with acetyl acacic acid. Their infra-red spectra are superimpossible.

Deacetylation of the acetate : The acetate (60 mg) was refluxed with methyl alcoholic potassium hydroxide (10%) for three hours on a water bath. Half of the methyl alcohol was distilled off and the mixture poured in water. It was then extracted with ether and the alkaline solution acidified with hydrochloric acid when the free genin was obtained as a colourless precipitate. It was filtered and washed free of hydrochloric acid. The product was crystallised from a large volume of methyl alcohol as colourless needles m. p. 271-272° (Cf. Acacic acid m. p. 280-81°). No depression was noted when mixed melting point was taken with acacic acid.

Selenium dioxide oxidation : Acetate (100 mg) in acetic acid (100 cc) was mixed with selenium dioxide (100 mg) and refluxed for 3 hours. On recrystallisation the product had m. p. 210-12° and gave deep yellow colour with tetranitromethane. In Ultraviolet spectra it showed two maximas at 255 mμ and 265 mμ.

Methyl ester of the genin : The genin (1 g) was suspended in ether (500 ml) and an excess of an ethereal solution of diazomethane added to it. The reaction mixture was kept overnight, filtered and the excess diazomethane and ether evaporated off. The residue was crystallised from methyl alcohol as colourless needles m. p. 260-62°.

Oxidation of methyl ester : The methyl ester (0·2 gm) was dissolved in pyridine (4 ml) and added to chromium trioxide (0.3 gm) in pyridine (8 ml). The solution was kept at room temperature for 24 hours, poured into ice cold water and excess chromic acid decomposed with HCl and sodium sulphite. The product was extracted with ether. The ether extract was washed with 5% HCl, water and dried. Evaporation of ether gave the product (0·12 gms) m. p. 338-42°.

Found C 76. 44%; H 9·13%

Calc: $C_{30}H_{44}O_4$. C76·88%; H, 9·46%.

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CHEMICAL EXAMINATION OF THE SEED MUCILAGE FROM THESPESIA POPULNEA SOLAND

By

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SUMMARY

Successive hydrolysis of the mucilage obtained from the fresh air dried seeds of *Thespesis populnea* Soland, with 3 percent oxalic acid and 2N sulphuric acid yielded arabinose, xylose, rhamnose and aldobionic acid, galactose, xylose, rhamnose, arabinose and galacturonic acid respectively.

Kaempferol and its glycoside (originally designated as populnetin and populnin respectively) along with herbacetin were reported[1,2,3] to have been isolated from the petals and the flowers of *Thespesia populnea* Soland. Recently[4] another new colouring matter, provisionally named 'Thespesin', $C_{19}H_{20}O_5$, has been isolated from the air dried fruits and on the basis of the data available a provisional structure of a flavanone with three hydroxyl groups and a C_4H_9—chain in unlocated positions has been assigned to it. The fatty constituents of the dark red oil from the seeds has already been investigated[4,5] and the present communication deals with the results of examination of the seed mucilage.

The buff coloured mucilage (yield 10 per cent; eq. wt., 1150) on ignition gave 5 per cent of a greyish white ash which showed the presence of iron, calcium and magnesium. Hydrolysis of the mucilage with 3 per cent oxalic acid yielded arabinose, xylose, rhamnose, and aldobionic acid. The unhydrolysed residue left after the oxalic acid hydrolysis was hydrolysed with 2 N sulphuric acid which resulted in galactose, xylose, rhamnose, arabinose and galacturonic acid.

EXPERIMENTAL

500 gms. of the powdered and dried seeds were successively extracted with petrol ether (40—60) and acetone to remove the fatty constituents and the colouring matter. The residual plant material was extracted with 3 per cent acetic acid and the extract filtered through muslin. The filtrate was poured in a large volume of alcohol and the precipitated mucilage was subsequently purified four times by a repetition of the process. The purified and the dried mucilage was successively refluxed with petrol other, benzene chloroform and methanol to remove last traces of impurities. (yield 50 gms.; found C, 38.2; H, 7.0 per cent: eq. wt. by itration, 1150).

Hydrolysis with oxalic acid : 40 gms. of the mucilage was hydrolysed with 3 per cent oxalic acid on a water bath for 20 hours and the reaction mixture was filtered from the unhydrolysed material. The solution was neutralised with barium carbonate, filtered and the filtrate concentrated to a small volume aud diluted with an excess of methanol whereby the barium salt of the sugar acid was precipitated out (found Ba, 15.8; required for aldobionic acid, 16.1 per cent).

The filtrate showed the presence of xylose, arabinose and rhamnose on paper chromatography (solvent: butanol : acetic acid: water, 4:1:5; spraying agent, aniline phthalate).

Hydrolysis with 2N sulphuric acid : The unhydrolysed residue from above was heated with 2N sulphuric acid on the water bath for 20 hours. The reaction mixture was filtered and worked up in the same manner as in the case of oxalic acid hydrolysis yielding the Barium salt as a precipitate.

Found : Ba, 26.8 ; required for galacturonic acid, 269. per cent).

The sugars present in the filtrate were identified as galactose, xylose, rhamnose and arabinose through chromatography.

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SOME MINOR CONSTITUENT ACIDS OF LAC RESIN

By

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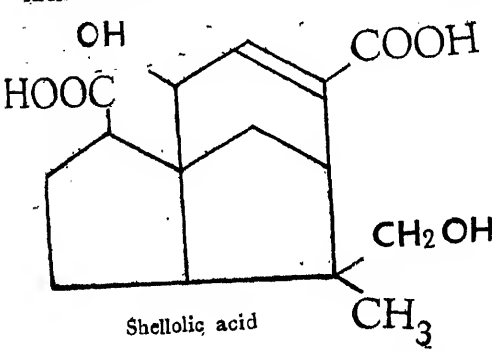
ABSTRACT

Lac resin is believed to be composed of a mixture of aliphatic and hydroaromatic hydroxy acids which are present as cross-linked polyesters. The presence of aleuritic acid and its isomer (30-35%), butolic acid (5-8%), shellolic acid (3-4%), *epi*-shellolic acid, and jalaric acid (~25%) have been proved with certainty. The present paper describes the isolation and identification, by various chromatographic techniques, of a few straight chain aliphatic acids such as tetra-, hexa- and octa-decanoic acids, a mixture of tetradecenoic acids, hexa- and octa-dec-9-enoic acids, 16-hydroxyhexadecanoic acid and 16-hydroxyhexadec-*cis*-9-enoic acid. The presence of 6-ketotetradecanoic acid has also been indicated.

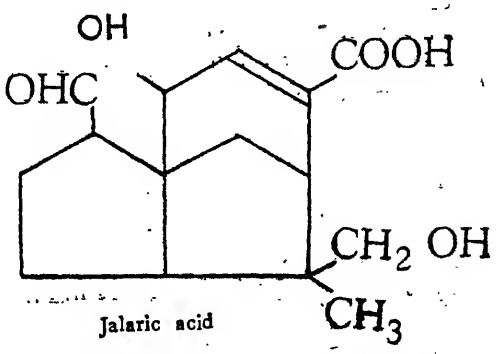
Lac resin is the main constituent of shellac and is always associated with a dye, a wax and an odoriferous principle. It is a mixture of several constituents of different molecular complexities and the constituents are believed to be mainly a mixture of cross-linked polyesters largely derived from aliphatic and hydroaromatic hydroxy acids. The constituent acids from the resin hydrolysate have been the subject of extensive investigation for a pretty long time and some definite results have been achieved in identifying the major ones. The presence of myristic[1], palmitic[1] mono-[2], di-[2], tri-(aleuritic and its isomers)[3,4] and tetra-hydroxyhexadecanoic[4] (kerrolic) acids, of butolic[5], shellolic[6], *epi*-shellolic[7,8], aldehydic[9,10] (jalaric) and unsaturated hydroxy[1] acids have been reported. The structure of aleuritic acid[6] has been proved to be 9,10,16-trihydroxyhexadecanoic acid,

$$[OH.CH_2.(CH_2)_5.CH(OH).CH(OH).(CH_2)_7COOH].$$

The structure [11] (a cedrenoid sesquiterpene) and absolute configurations of shellolic[7,12],



Shellolic acid



Jalaric acid

and jalaric[8] acids have been deduced recently. Butolic acid has been shown to be 6-hydroxytetradecanoic acid and the structure has been confirmed by synthesis,[13]

$$[CH_3.(CH_2)_7.CH(OH).(CH_2)_4COOH]$$

From the present day knowledge it may be suggested that the resin hydrolysate contains aleuritic and isomeric acids (30-35%), shellolic acid (3-4%), jalaric acid (~25%), butolic acid (5-8%) and undesignated acids (30-35%).

It will be evident from the above that 30-35% of the constituent acids still remain unidentified and undesignated which require further investigation.

The present paper deals with the isolation and identification of some minor component acids of lac resin.

Dewaxed decolourised lac has been hydrolysed with normal solution of ethanolic caustic potash at room temperature and the acids recovered by passing the solution through cation exchange resin and evaporating in the rotary film evaporator at a temperature $\geq$ 40°. The mixture of acids was then converted to methyl esters by methanolic hydrogen chloride.

The mixed esters were chromatographed on neutral alumina by eluting successively with ether, a mixture of ether and methyl alcohol and methyl alcohol. Thus nine fractions (A_1 to A_9) were collected. Examination of the fractions by Thin Layer Chromatography (TLC) against standards and by Gas Liquid Chromatography (GLC) revealed that the fractions have been obtained in the order of increasing polarity.

Fraction A_1 mostly consisted of non-hydroxy esters and was refractionated into four fractions on silicic acid with increasing proportion of ether in benzene. The non-hydroxy esters were concentrated in the first one which was again fractionated, according to the method of De Vries,[13] on silicic acid impregnated with silver nitrate with increasing proportion of ether in petroleum ether (40-60°) and the pure straight chain saturated (0.6%) and unsaturated (0.2%) esters collected.

Examination of the saturated esters on GLC revealed it to be a mixture of tetradecanoic (30.8%), hexadecanoic (64.8%) and octadecanoic (4.4%) esters, while the unsaturated esters were found to be a mixture of tetradecenoic (26.2%), hexadecenoic (27.1%) and octadecenoic (46.7%) esters.

The mixed unsaturated esters were further fractionated into individual pure ester fractions by means of perparative GLC. The cleaved products obtained by oxidative degradation[14] were identified by GLC. The hexadecenoic and octadecenoic esters were found to have the double bond largely in the 9:10 position. The tetradecenoic ester was found to be a mixture of esters with the double bond in the 9 : 10, 8 : 9, 7 : 8, 6 : 7 and 5:6 positions.

Fraction A_2 mainly consisted of mono- and di-hydroxy esters. The fraction was rechromatographed on silicic acid column with increasing proportion of ether in benzene and five fractions were collected. Third fraction was the largest ($\sim$5% of total lac esters) and mainly a mixture of 6-hydroxy C_{14} (butolate) and 16-hydroxy C_{16} esters. It was acetylated, treated with urea and the non-adducted 6-acetoxy ester was separated from the adducted 16-acetoxy esters. The latter after saponification was oxidised to give the dibasic acid. Examination of the diester by GLC showed it to be a mixture of saturated and unsaturated C_{16} esters.

For isolation and identification of the pure 16-hydroxy esters, lac acid esters were fractionated by urea. The adducted esters were acetylated and refractionated with urea. The non-adducted ester portion was found to mainly consist of the butolate. The adducted esters were examined by GLC and TLC (on silver nitrate sprayed plates) and were found to be mainly saturated and unsaturated non-acetoxy and acetoxy esters.

The adducted esters were then refractionated over silicic acid impregnated with silver nitrate[15] by means of increasing proportion of ether in petroleum ether (40-60°). The fractions were monitored by the help of TLC and GLC and pure fractions were taken together giving ultimately six fractions. The first two were saturated and unsaturated non-acetoxy (i.e. non-hydroxy) esters while the

third and the fifth saturated and unsaturated 16-acetoxy esters respectively. The fourth and the sixth fractions were mixtures.

The third fraction was hydrolysed and the acid crystallised from ether by slow evaporation at room temperature. The acid had m. p. of 92-93° and was undepressed by authentic sample of 16-hydroxyhexadecanoic acid (mp. 93-94°). A mixture of diesters obtained from the oxidation of the natural and authentic acids showed only one peak on the GLC proving conclusively the identity of the natural acid as 16-hydroxyhexadecanoic acid, [$OH.CH_2.(CH_2)_{11}.COOH$].

The fifth fraction after hydrolysis was oxidised with potassium periodate and potassium permanganate according to Von Rudloff.[14] The main oxidised products were identified by GLC to be C_7 and C_9 dibasic acids, and 7-hydroxy heptanoic acid; these can only be obtained from 16-hydroxyhexadec-9-enoic acid, [$OH.CH_2.(CH_2)_5$-CH:$CH.(CH_2)_7$ $COOH$].

The natural acid was a liquid and did not show any absorbance near 10.4μ on IR indicating further that it is 16-hydroxyhexadec-*cis*-9-enoic acid. These 16-hydroxy acids represent 0.4 percent (saturated) and 0.7% (unsaturated) of the total lac acids.

The fourth fraction was found to be one major component associated with some other esters. The major component appeared to be different from the acids isolated earlier as there was no change in the carbon number (on GLC) before and after hydrolysis suggesting the absence of any hydroxyl group. A strong absorption was noticed near 5.78μ on the IR indicating the presence of some carbonyl function in the molecule. Since the carbon number of the ester corresponded with that of 6-ketotetradecanoate, it is believed that the acid might be 6-ketotetradecanoic acid, [$CH_3.(CH_2)_7.CO.(CH_2)_4COOH$].

The fractions A_3 & A_4, which constituted nearly 9% of total lac esters, were mainly composed of mono- and di-hydroxy esters along with some tri-hydroxy esters. These two fractions were combined and rechromatographed over silicic acid with increasing proportion of ether in benzene and four fractions were collected. The first three were a mixture of mono-, di- and tri-hydroxy esters while the fourth one di- and tri-hydroxy esters only.

The second one was the largest and after acetylation was once again fractionated over silicic acid with increasing proportion of ether in petroleum ether (40-60°) and nine fractions collected. The fourth one on standing gave crystals which were repeatedly washed with petroleum ether. The pure crystalline ester gave a single peak on GLC and a single spot on TLC. The acetoxy ester melted at 117-118° and the elementary analysis agreed with the formula $C_{19}H_{28}O_6$.

The acetoxy ester was converted to the acid and then to the methyl ester which showed one peak on GLC having a carbon number different from that of the acetoxy ester. This indicated the presence of hydroxyl group. The acid melted at 213-215° and the elementary analysis agreed with the formula $C_{15}.H_{22}O_5$ The IR spectrum showed the presence of unsaturation in the molecule and was in much agreement with that of shellolic acid.

Based on the above findings, it is believed that the acid might be an unsaturated monohydroxy dicarboxylic C_{15} acid having the shellolic acid nucleus.

During the investigation apart from the dihydroxy straight chain acids further indications have been obtained for the presence of trace amount of 14-hydroxy-

tetradecanoic acid and a few other lower homologues of it and of 6-hydroxy-tetradecanoic acid.

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ESTIMATION OF ALCOHOLIC CONSTITUTENTS AND ESTERS IN ESSENTIAL OILS AND SYNTHETIC MIXTURES PART XIV

By

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ABSTRACT

In the present investigation the work has been carried out on the applicability of the three formulae in the estimation of alcohol in essential oils and synthetic mixture. The authors have shown that the formula L_3 derived by them gives better results in comparison to the Ramanathan's formula L_2 in case the acetylated mixture is washed with brine solution containing sodium carbonate so as to be completely free from acid. Whereas the Cocking's formula L_4 and the formula L_5 are equivalent and give the same result when the acetylated product is washed with brine solution only.

During recent years the estimation of alcoholic constituents has attracted the attention of a number of essential oil chemists[2,4,5,6]. For estimation of alcoholic constituent in an essential oil the standard practice is to apply Cockings's formula,[3] the washing of acetylated oil being conducted with sodium chloride solution. The Indian Standard Methods of Tests[10] for Essential oils 326-1952 specify that the washing of the acetylated essential oil should also be carried out once with 50cc. of sodium chloride solution containing two per cent sodium carbonate. The completeness or washing operation should be verified by determination of its acid value which should be zero and then according to Ramanathan,[7] the following formula L_1 would be applicable for determination of free alcohol content.

$$a = \frac{M_a (S_2 - v_1)}{561{\cdot}04 - 0.4201 s_2} \quad \ldots \quad \ldots \quad \ldots \quad (L_1)$$

Consider a mixture containing a, b, c, d and k percent of alcohols A, ester B, acid C, hydrocarbon D and ketone K and let their respective molecular weights be M_a, M_b, M_c, M_d and M_k and the ester value of 100 percent pure acetate of alcohol A be V_a and that of ester B be V_b respectively. Further, let V_c be the acid value of pure acid C and v_1, v_3 and s_1 be the respective ester value, acid value and saponification value of the mixture before acetylation. Further, let v_2 and s_2 be the ester value and saponification value of the mixture after acetylation.

Then $a + b + c + d + k = 100$ .....(1)

$$b = \% \text{ of Ester B} = \frac{100 v_1}{V_b} = \frac{v_1 M_b}{561.04} \quad \ldots\ldots(2)$$

$$c = \frac{100\, v_3}{V_c} = \frac{v_3 M_c}{561.04} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(3)$$

After acetylation the proportion of acetate of A, to ester B, to acid C, to hydrocarbon D and to ketone *k* would be as follows :—

$$a\left(1+\frac{42.01}{M_a}\right) : b : c : d : k$$

$$\text{Therefore, } v_2 = \frac{a\left(1+\frac{42.01}{M_a}\right)V_a + bV_b}{a\left(1+\frac{42.01}{M_a}\right)+b+c+d+k} \quad \ldots\ldots(4)$$

It can be shown that $a = \frac{M_a(v_2 - v_1)}{561.04 - 0.4201v_1}$ ..... (L_2)

This is the well known Cocking's formula[3] first derived and suggested by him for estimation of an alcohol in an essential oil.

$$\text{Now } s_2 = (v_2 + v_4) = \frac{a\left(1+\frac{42{\cdot}01}{M_a}\right)V_a + bV_b + cV_c}{a\left(1+\frac{42{\cdot}01}{M_a}\right)+b+c+d+k}$$

$$= \frac{a(M_a + 42{\cdot}01)V_a + 100v_1M_a + 100v_3M_a}{100M_a + 42{\cdot}01a}$$

or $100M_a(v_2 + v_4) + 42{\cdot}01a\ (v_2 + v_4) = a\ (M_a + 42{\cdot}01)\ V_a + 100v_1M_a + 100v_3M$

or $a\ (56104a - 420\ |\ (v_2 + v_4)) = 100M_a\ ((v_2 + v_4) - (v_1 + v_3))$

$$\text{or \% of alcohol} = a = \frac{M_a(s_2 - s_1)}{561{\cdot}04 - 0{\cdot}4201s_2} \quad \ldots\ldots(L_3)$$

As is evident mathematically the two formulae L_1 and L_3 are equivalent and would give identical results provided during the acetylation no interfering action occurs and the only chemical change taking place is the acetylation of free alcohol present and further during the washing operation of the acetylated product none of the constituents of the essential oil are removed.

In case, the acetylated product is thoroughly washed with sodium chloride solution containing sodium carbonate so as to completely free the acetylated product from organic acids, the proportion of acetate of A to ester B, acid D and ketone K would be as follows :—

$$a\left(1+\frac{4201}{M_a}\right) : b : d : k.$$

$$v_2 = \frac{a(M_a + 42{\cdot}01)V_a + 100v_1M_a}{100M_a + 42{\cdot}01a - cM_a}$$

Therefore, $100M_a v_2 + 42{\cdot}01\, a\, v_2 - cM_a v_2 = a(M_a + 42{\cdot}01)V_a + 100\, v_1 M_a$

or $a\left[(M_a + 42{\cdot}0)V_a - 42{\cdot}01\, v_2\right] = 100M_a v_2 - cM_a v_2 - 100\, v_1 M_a$

or $a(56104 - 42{\cdot}01\, v_2) = 100M_a\left[(v_1 - v_1) - \frac{cv_2}{100}\right]$

Hence $a = \dfrac{M_a\left[v_2\left(1 - \frac{c}{100}\right) - v_1\right]}{561{\cdot}04 - 0{\cdot}4201\, v_2}$ ..... (L_4)

When the acetylated product is completely freed from organic acids by washing with sodium chloride solution containing sodium carbonate Ramanathan's formula L_1 and Cocking's formula L_2 become identical as saponification value S_3 becomes identical with ester value v_2.

Ramnathan[7] has referred to two methods of washing the acetylated essential oil while there can be three procedures differing in details and results. The essential oil after acetylation is heated with 50 *c.c.* of water at moderate temperatures for 0·25 to 0·50 hour and after cooling the aqueous layer is separated and the acetylated oil may then be further processed in one of the following ways :

1. It is washed with saturated sodium chloride solution till the acetylated oil is neutral.
2. Acetylated oil after removal of aqueous layer is washed successively with 50 *c.c.* of brine, 50 *c.c.* of brine containirg 1 *g.* of sodium carbonate in solution, 50 *c. c.* of brine and finally with 20 *c. c.* of water.
3. The acetylated oil is washed with saturated brine containing 2 per cent sodium carbonate in solution till the wash liquor is alkaline towards litmus and finally at least with 20 *c. c.* of water so that the aqueous washing is neutral towards litmus.

The procedure 1 is the best and the only disadvantage with it is that the removal of acetic acid may not be fast enough. Zuthshi and Sadgopal[11] have followed procedure 1 in their studies of various samples of Indian vetiver oils and Ramnathan has advocated this procedure for washing of acetylated essential oils containing free acids and the application of formula L_3.

In procedure 2 the removal of acetic acid is easy and quick and one washing with brine solution containing sodium carbonate serves to remove the acetic acid formed from acetic anhydride but does not remove the acids originally present in the essential oil and any of the formula L_2 or L_3 would be equally applicable. The procedure 2 is laid down in British Standard Method[1,8] of testing of Essential Oils. The third procedure would result in the complete removal of the acids originally present in the essential oil and under this condition formula L_4 would be applicable and not the formulae L_1 as suggested by Ramnathan.

In view of the different procedures advocated in literature by authors for

washing the acetylated essential oil and the formula applicable under the conditions of washing it was considered desirable to study this problem.

In the experimental part details of verification of the above three formulae employing the following systems under different circumstances are given.

1. Binary mixtures of phenyl ethyl alcohol and lauric acid.
2. Sandalwood oil and lauric acid, and
3. Benzoic acid, benzyl acetate and menthol.

EXPERIMENTAL

The physico-chemical properties of chemicals used in the investigation are given in Table 1.

TABLE 1

Physico-Chemical Constants

Particulars	Sandal wood oil	Phenyl-ethyl alcohol	Benzyl acetate	Menthol	Benzoic acid	Lauric acid
1. Specific gravity.	0·9680 at 30°C	1·0199 at 25°C	1·069 at 20°C	...	...	...
2. Refractive Index.	1·5060 at 30°C	1·5320 at 16°C	1·5043 at 34°C	...	...	...
3. Optical rotation (α_D)	—17·9°	...	...	...	...	...
4. Acid value	2·12	...	...	...	459·42	281·08
5. Ester value	8·06	...	373·6	...	...	...
6. Ester value after acetylation.	207·0	342·5	...	282·8	...	...
7. % purity	92·05 (Santalol%)	100·3	99·9	99·99	100·0	100·0
8. % acid as lauric acid	0·75	...	...	...	...	...

Preparation of Mixtures :

Mixtures were prepared and stored in amber coloured bottles before use.

Acetylation of Mixtures :

Mixture (10 *c. c.*), redistilled A. R. acetic anhydride (20 *c. c.*) and fused sodium acetate A. R. (1 gm) were taken in a 100 *c. c.* acetylation flask fitted with one meter long standard joint air condenser. The contents of the flask were heated gently (just simmering) on a sand bath for 2 hours after which the flask was cooled, air condenser washed with 100 *c. c.* of warm distilled water 45-50°C and the washings collected in the acetylation flask. It was then kept aside for 30 minutes to decompose the excess of acetic anhydride present. The acetylated mixture was washed in a separating funnel with saturated brine solution several times till the mixture was practically neutral towards litmus and finally twice with distilled water using 50 *c.c.* for each washing. The acetylated mixture was dried over anhydrous sodium sulphate (1 gm.) for 18 hours, filtered and analysed.

The analytical details of phenyl ethyl alcohol and lauric acid system using modified technique are given in table 2 and the percent of phenyl ethyl alcohol found using formulae L_3 and L_2 are practically the same.

TABLE 2

Analytical Details of Phenyl Ethyl Alcohol & Lauric acid System

Mixture No.	I	II	III	IV
1. *% Composition of mixtures.*				
i. (*a*) Phenyl ethyl alcohol ...	99·20	93·23	84·89	70·50
(*b*) Lauric acid ...	0·899	6·77	15·10	29·49
ii. Molar ratio of lauric acid to phenyl ethyl alcohol.	1:203·6	1:22·58	1:9·22	1:3·92
2. *Before Acetylation* (*Mean*).				
(*a*) Acid value ...	2·25	18·78	41·96	81·61
(*b*) Sap. value ...	2·25	18·78	41·963	81·60
(*c*) Ester value ...	0	0	0	0
3. *After acetylation*				
(*a*) Acid value ...	1·52	14·25	32·03	65·35
(*b*) Saponification value ...	314·1	339·24	333·7	323·6
(*c*) Ester value ...	339·6	325·0	301·6	
4. *% Alcohol found from*				
i. Formula L_3 ...	99·10	93·5	84·65	69·50
ii. Formula L_2 ...	99·16	93·55	84·75	96·71

Next, the six mixtures of lauric acid and sandalwood oil were examined as detailed in table 3. Their saponification value before and after acetylation was determined by the modified technique as previously described[4]. The details regarding the determination of saponification value before and after acetylation are given in table 4 and 5 respectively. The experimental results prove beyond

TABLE 3

Details of sandal wood oil & lauric acid system

Mixture No.	I	II	III	IV	V	VI
1. % *Composition*.						
(*a*) Sandal wood oil	98·02	95·03	89·80	79·07	98·0	94·96
(*b*) Lauric acid	1·98	4·97	10·2	20·93	2·0	5·04
2. % Composition (*calculated*).						
(*a*) Santalol	90·23	87·45	82·67	72·78	90·21	87·41
(*b*) Acid*	2·70	5·68	10·88	21·52	2·74	5·74
3. *Before acetylation.*						
(*a*) Acid value	7·15	15·4	29·5	59·1	7·25	15·3
(*b*) Saponification value.	13·7	23·35	37·4	67·8	13·53	23·76
(*c*) Ester value	6·55	7·95	7·9	8·7	6·28	8·46
4. *After acetylation.*						
(*a*) Acid value	6·0	12·6	25·15	50·2	6·60	13·71
(*b*) Saponification value	209·0	212·5	214·2	220·4	209·3	213·1
(*c*) Ester value	203·0	199·9	189·05	170·2	202·7	199·39
5. % *Composition* (*found*)						
(*a*) (*i*) Santalol (Formula L_3)	90·94	88·32	82·72	72·19	91·12	88·04
(*ii*) Santalol (formula L_2)	91·18	88·57	83·5	72·66	90·91	83·11
(*b*) Acid as lauric acid	2·55	5·50	10 52	21·10	2·59	5·47

*Total acid as lauric acid.

TABLE 4

Benzoic acid, Benzyl Acetate and Menthol System

Particulars/Mixture	1	2	3	4	5	6	7
1. % *Composition*							
(*a*) Benzoic acid	2·14	3·74	6·50	8·0	8·72	12·16	17·26
(*b*) Benzyl acetate	31·86	69·86	37·36	61·33	50·45	71·16	54·58
(*c*) Menthol	65·70	26·40	56·15	30·62	40·78	16·66	27·94
2. *Before acetylation* :							
(*a*) Acid value (Mean).	9·7	17·5	30·4	37·4	40·0	57·7	80·7
(*b*) Saponification value (Mean)	127·3	275·6	170·3	267·2	227·6	322·7	286·0
(*c*) Ester value (Mean)	117·6	258·1	139·9	229·8	187·6	265·0	205·3
3. *After Acetylation.*							
(*a*) Acid value (Mean)	0	0	0	0	0	0	0
(*b*) Saponification value (Mean)	305·3	342·7	313·4	338·7	323·6	351·2	335·9
4. % *Composition (Found).*							
(*a*) Benzoic acid	2·13	3·80	6·65	8·14	8·70	12·56	17·57
(*b*) Benzyl acetate	31·48	69·10	37·45	61·51	50·21	70·93	55·0
(*c*) Menthol							
(*i*) Formula L_2	64·25	25·13	52 07	26·68	35·30	10·77	18·56
(*ii*) Formula L_3	67·77	31·70	63·13	40·56	50·42	32·57	48·60
(*iii*) Formula L_4	65·42	26·81	55·55	30·38	39·63	15·90	26·63
5. % *Deviation in*:							
(*a*) Benzoic acid	−0·01	+0·06	+0·15	+0·14	−0·02	+0·40	+0·31
(*b*) Benzyl acetate	−0·38	−0·76	+0·09	+0·13	−0·24	−0·23	+0·42
(*c*) Menthol :—							
(*i*) Formula L_2	−1·45	−1·27	−3·08	−3·94	−5·48	−5 89	−7·38
(*ii*) Formula L_3	+2·07	+5·30	+6·98	+9·94	+9·68	+15·91	+21·66
(*iii*) Formula L_4	−0 28	+0 41	−0·60	−0·22	−1·15	−0·76	−1·31

doubt that the two formulae L_3 and L_2 involving respectively ester value and saponification value of the essential oil before and after acetylation give identical results and are equivalent.

Seven mixtures of benzoic acid, benzyl acetate and menthol, were prepared and analysed by modified techique for the determination of saponification value and acid value before acetylation. These mixtures after acetylation were washed with sodium chloride solution containing 4 percent sodium carbonate so as to make it completely free from benzoic acid. The results detailed in table 6 indicate that under this condition of washing, only formula L_4 gives correct result while the formula L_3 gives widely different results and the deviation from the actual values increases with the acid content of the mixture. The application of Ramanathan's formula L_1 which becomes identical with Cocking's formula L_2 under these conditions give results quite similar to formula L_3.

CONCLUSION

For estimation of alcoholic constituent of an essential oil containing an appreciable amount of free acids Cocking's formula L_2 or the formula L_3 are equally applicable provided after acetylation the washing of acetylated oil is carried out with sodium chloride solution only.

Further, in case washing of the acetylated essential oil is performed with sodium chloride solution containing sodium carbonate and the original acids of the essential oil are completely removed during washing the theoretically derived formula L_4 alone is applicable and not the formula L_1 suggested by Ramanathan.

The washing of the acetylated essential oil is best carried out with saturated sodium chloride solution.

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ISOLATION AND STUDY OF THE MUCILAGE FROM THE FRUIT OF CORDIA MYXA LINN.

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ABSTRACT

The defatted pulp of the Cordia myxa fruit was extracted with water and the mucilage precipitated with ethanol, repeating this procedure several times to obtain a product with minimum ash content. Preliminary analysis of the purified mucilage gave methoxyl content 5.28%; uronide content 15.7% and acetyl ester 4.17%. Hydrolysis of the mucilage and paper chromatographic examination of the hydrolysate revealed the presence of D-galactose, L-arabinose, D-xylose, D-galacturonic acid and an unidentified sugar having a low yield. Quantitative estimation of the sugars was carried out by paper chromatography followed by elution and periodate oxidation giving D-galactose 31.8%, D-xylose 12.9%, and L-arabinose 19.83%. Repeated methylation with dimethyl sulphate and sodium hydroxide followed by Purdie's reagents gave a methylated product which was fractionated into four fractions. These fractions on hydrolysis gave 2, 3, 4, 6-tetra-O-methyl-D-galactose ; 2, 3, 6-tri-O-methyl-D-galactose; 2, 4, di-O-methyl-D-galactose; 2-O-methyl-L-arabinose ; 2, 3, 4, -tri-O- methyl-D-xylose; and 2, 3,-di-O-methyl-D-galacturonic acid. On the basis of these a structure for the mucilage has been suggested.

Cordia myxa Linn. known as Lassora in Hindi, belongs to the natural order Boraginaceae. The fruit is reported for its medicinal importance.[1,2]

The present work was undertaken with a view to make a detailed study of the mucilage which forms a major part of the fruit.

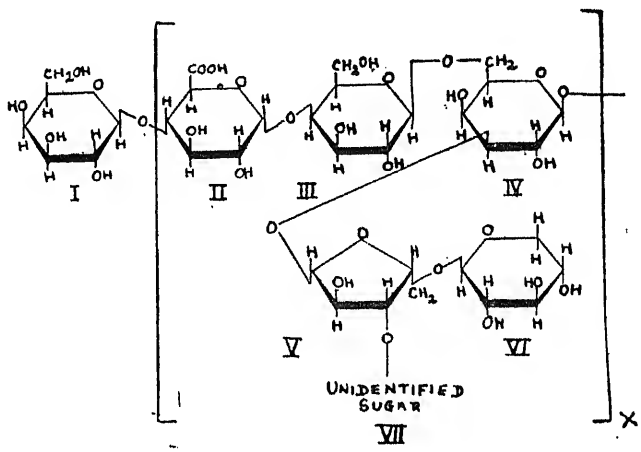
The mucilage has been extracted with acidulated water and precipitated with ethanol, repeating this procedure several times for purification. On preliminary analysis the purified mucilage gave the following results:— methoxyl 5.28%, acetyl 4.17%, and uronide 15.7%. Complete hydrolysis of the mucilage with 2N sulphuric acid and paper chromatographic examination of the hydrolysate revealed the presence of galactose, arabinose, xylose, galacturonic acid and an unidentified sugar. These sugars were estimated quantitatively by chromatography followed by elution and periodate oxidation of the elute and found to be galactose 31.8%, arabinose 19.83% and xylose 12.9%.

Methylation with dimethyl sulphate and sodium hydroxide[3] gave a product (OMe 9.98%) which was remethylated with Purdie's reagents.[4] On fractionation with chloroform: petroleum ether mixture, four fractions were obtained. On hydrolysis of these fractions and identification the presence of 2, 3, 4, 6-tetra-O-methyl-D-galactose ; 2,3,6-tri-O-methyl-D-galactose ; 2, 4-di-O-methyl-D-galactose ; 2-O-methyl-L-arabinose; 2,3,4-tri-O-methyl-D-xylose and 2, 3, di-O-methyl-D-galacturonic acid was revealed.

2, 3, 4, 6-Tetra-O-methyl-D-galactose has been identified in only one fraction in minute quantities. As only C_1 is free for linkage this tetra methyl galactose must be present as the terminal group I in the proposed structure. 2,3,6-Tri-O-methyl-D-galactose III having C_1 and C_4 unmethylated shows these to be

the points of linkage and has been shown in the repeating unit. 2, 4,-Di-O-methyl-D-galactose IV having C_1, C_3 and C_6 as free positions forms the branching point in the structure, linked to the repeating unit, III and V respectively. 2, 3,-Di-O-methyl-D-galacturonic acid has been identified as the only methylated galacturonic acid derivative and has been shown as molecule II linked through its C_1 and C_4 to the main chain. Arabinose and xylose form labile groups in the mucilage and have been thus assigned the position in the side chain. This is in fairly good agreement with the percentage of sugar and sugar acids determined quantitatively.

STRUCTURE



EXPERIMENTAL

Unless otherwise stated, specific rotations were measured in chloroform; paper chromatographic separations were carried out on Whatman No. 1 paper using the following solvents (A) butanol: ethanol: water (40:10:50)[5] (B) butanol; ethanol: ammonia: water (40:10:1:49) (C) butanol:acetic acid:water (5:1:4). The spray reagents used were aniline hydrogen phthalate ; silver nitrate in acetone and ethanolic sodium hydroxide; naphthoresorcinol and trichloracetic acid.

Isolation and purification of the mucilage:—The semi-ripe fruit was crushed gently to separate the pulp from the unbroken seeds. The entire mass was washed with water, dried and extracted exhaustively with petroleum ether, acetone, alcohol and ether. The pulp and seeds were then suspended in water acidulated with acetic acid and stirred mechanically for 36 hrs. Each batch of seeds and pulp were thus extracted three to four times to ensure maximum extraction of the mucilage. The extract was filtered and added slowly, with stirring to 90% ethanol. The crude precipitated mucilage was filtered, washed with ethanol and ether and dried in vacuum. This procedure was repeated several times till the product gave a negative test for nitrogen and the ash content was reduced to a minimum. The purified mucilage had $(\alpha)^{30}D$ + 107·07° in water; methoxyl content[6] 5·28%; acetyl ester[7,8] 4.17%; and uronide content[9] 15.7%.

Hydrolysis and identification of the sugars in the hydrolysate:—0.05gm of mucilage was hydrolysed with 2N sulphuric acid for 48hr. The hydrolysate was neutralised with barium carbonate, filtered and concentrated under reduced

pressure. Paper chromatograms of the hydrolysate were run in solvents (B) and (C) and each sprayed with a different spray reagent.

The Rf values were measured and compared with those given in literature.[5,10]

Solvent (B)		Solvent (C)		Sugar identified
Rf found	Rf. given	Rf. found	Rf. given	
0.085	0.09	0.15	0.16	galactose
0.14	0.145	0.20	0.21	arabinose
0.165	0.170	0.27	0.28	xylose
0.024	—	0.135	0.14	galacturonic acid
0.625	—	0.71	—	unidentified sugar

Another chromatogram with spots of the hydrolysate and reference sugars galactose, arabinose, xylose and galacturonic acid was run in solvent (A). The spots of the hydrolysate sugars corresponded with those of the reference sugars.

Quantitative estimation of the monosaccharides in the hydrolysate:- 0.05 gm of purified mucilage was refluxed with 1.5N sulphuric acid and neutralised with barium carbonate. The filtrate was made up to 25 ml and a known volume applied on a paper chromatogram along with guide spots. On developing in solvent (B), the strips corresponding to the guide spots were cut and eluted. The elutes were oxidised with periodate[11] and the quantity of monosaccharides determined. Blanks were run simultaneously.

Found:—Arabinose 19.83%, xylose 12.9% and galactose 31.8%.

Methylation:—The mucilage (3 gms) was dissolved in water (20 ml), dimethyl sulphate (30 ml) and 40% sodium hydroxide (60 ml) were added with constant stirring during 8 hrs. The temperature was maintained at 30°C during addition. The mixture was remethylated and the thick methylated syrup concentrated and dissolved in acetone. The acetone solution was remethylated thrice and the concentrated syrup extracted exhaustively with chloroform in a liquid-liquid extractor (downward displacement type) for 20 hrs. The chloroform extract was dried over anhydrous sodium sulphate and distilled under reduced pressure. The methylated product (OMe 9.98%) was methylated further by Purdie's reagents[4] and the methylated mucilage fractionated by boiling it under reflux with mixtures (10 ml) of chloroform and light petroleum. Thus, four fractions were obtained.

Fractions	$CHCl_3$: Petrol	OMe%	$(\alpha)D^{30}$
A	1.5 : 8.5	11.78	+112.5°
B	2 : 8	13.16	−27.4
C	2.5 : 7.5	Low yield	+115.7°
D	insoluble in either	in traces.	—

Hydrolysis and identification of the methylated products :

The fractions A, B and C obtained on fractionation were hydrolysed with 1N sulphuric acid and methanol at 100° for 24 hrs. The hydrolysates were neutralised by barium carbonate and filtered. The filtrates were concentrated in vacuum and spots applied on paper chromatograms along with a spot of 2,3,4, 6-tetra-O-methyl-D-glucose. The chromatogram was developed in solvent (A) and sprayed with aniline hydrogen phthalate The Rg values (distance travelled by the methylated sugar divided by the distance moved by 2,3,4,6-tetra-O-methyl-D-glucose) were calculated and compared with those given in liteature.[12]

Thus

Rg found	Rg given	Sugar identified
Fraction A		
(1) 0.09	—	2-3-di-O-methyl-D-galacturonic acid.
(2) 0.705	0.71	2,3,6-tri-O-methyl-D-galactose
(3) 0.82	—	Unidentified methylated sugar
(4) 0.94	0.94	2,3,4-tri-O-methyl-D-xylose
Fraction B		
(1) 0.102	—	2, 3-di-O-methyl-D-galacturonic acid.
(2) 0.405	0.41	2,4-di-O-methyl-D-galactose
(3) 0.715	0.71	2,3,6-tri-O-methyl-D-galactose
(4) 0.827	—	Unidentified methylated sugar
(5) 0.874	0.88	2,3,4,6-tetra-O-methyl-D-galactose.
Fraction C		
(1) 0.11	—	2,3-di-O-methyl-D-galacturonic acid
(2) 0.37	0.38	2-O-methyl-L-arabinose
(3) 0.71	0.71	2,3,6-tri-O-methyl-D-glactose
(4) 0.83	—	Unidentified methylated sugar

The methylated uronic acids appeared as crimson red spots on the chromatogram sprayed with aniline hydrogen phthalate[13].

Identification of the methylated uronic acid was carried out by the following method.

Since all the fractions contained the uronic acid Rg. 0.11, only fraction A was used. Fraction A after hydrolysis was treated with barium hydroxide and heated at 100° for 5 hrs, neutralised by carbon dioxide and extracted with ether. The aqueous extract on concentration yielded the barium salt of the acid.

The barium salt was extracted with a small quantity of methanol to remove any traces of barium carbonate[14]. The barium salt of the methylated uronic acid was converted to the corresponding acid by removal of barium ions with Amberlite IR 120 (H)[15]. The methylated acid was dissolved in dry ether and the solution was added dropwise over a period of one hour to a stirred suspension of lithium aluminium hydride in ether. The mixture was gently heated under reflux for 2 hrs. cooled and excess lithium aluminium hydride decomposed by cautious successive additions of ethyl acetate and water[16]. After filtration it was concentrated and hydrolysed by 80% methanolic hydrochloric acid for 12 hrs. and further heated at 100° after removal of methanol. After neutralisation, filtration and concentration, the solution was examined paper chromatographically in solvent (A). A spot corresponding to 2,3-di-O-methyl-D-galactose Rg 0.45 was obtained (given Rg 0.46)[15].

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CHEMICAL EXAMINATION OF THE MUCILAGE OF THE SEEDS OF HYGROPHILA SPINOSA

By

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SUMMARY

The mucilage isolated from the seeds of Tal Makhana (Hygrophila spinosa) was found to have : methoxyl 3·00%; acetyl 1.38% pentoses 17·093% ; equivalent weight 2231 and uronide content 33·42%. On hydrolysis with 2 N sulphuric or 98-100% formic acid an oligosaccharide (Rf. 0·048) and xylose were obtained. The oligosaccharide contains urorie acid and is such that it resists further hydrolysis.

The mucilage of Tal Makhana (Hygrophila spinosa) was isolated by extraction with water and precipitation with ethanol. Purification of the mucilage was done by dissolving it in water and precipitation with ethanol and repeating this process till the ash content was reduced to a minimun.

Preliminary analysis of the purified mucilage gave pentoses 17.093%, equivalent weight 2231, methoxyl 3·00%, acetyl 1.38%, uronide content 33.42%.

On hydrolysis with 2N sulphuric acid (18hrs) xylose (Rf 0.18) and an oligosaccharide (Rf 0.043), butanol: ethanol: water: ammonia (45: 5: 49: 1) were obtained; the same two products were obtained when the mucilage was hydrolysed with formic acid (98-109%; 7hrs) or N sulphuric acid (30 hrs). The oligosaccharide (Rf 0.048) also contains the uronic acid part of the mucilage and resists hydrolysis by all the above reagents. A detailed examination of the oligosaccharide is in progress.

EXPERIMENTAL

Isolation and purification of the mucilage :

The powdered seeds were defatted with petroleum ether and then suspended in water, acidified with acetic acid and stirred for 36 hours. Each batch of eeds was thus extracted three to four times so as to dissolve the maximum amount of the mucilage. The aqueous extracts were filtered, first through cloth and then shrough filter paper. The mucilage was precipitated by addition of ethanol. The precipitate was filtered, washed with absolute ethanol and ether and dried in vacuum.

The mucilage was purified by dissolving it in water and precipitating with ethanol and repeating this process till the ash content was reduced to a minimum.

Pentosan content of the mucilage :

Purified mucilage was refluxed with hydrochloric acid (12%, 100 ml) in a flask on a water both at 100° and the distillate collected in a graduated cylinder, when 30 ml. of HCl (12%) was added from the dropping funnel. The process was continued till a drop of distillate gave no pink colour with aniline acetate paper. This stage was reached after collection of 330 ml. of the distillate. It was then treated with an excess of phloroglucinol solution, when dark green precipitate of furfural phloroglucinide began to deposit. The liquid was then made up to 400 ml. with HCl (12%) and allowed to stand overnight. It was filtered through a weighed sintered crucible. The precipitate was washed with water (150 ml). It was then dried in an oven for four hours and weighed. From the

increase in weight the amount of furfural was calculated to be 10.94% which corresponded to pentosan 15.05% and pentoses 17.093%.

Methoxyl :

Methoxyl percentage of the mucilage was determined by the method of Belcher, Fildes and Nutten.[1]

Found Methoxyl = 3.00%.

Acetyl group by saponification :

The Acetyl group was determined by the method of Wiesenberger[2] as described by Belcher[3].

Found acetyl = 1.38%.

Uronide content :

The uronide content of the mucilage was determined by the semi-micro method of Barker, Foster, Siddiqui and Stacey[4].

20 mg of the sample was taken in the dry reaction flask along with 3 ml. of 19% HCl. A stream of carbon dioxide free nitrogen was passed into reaction flask before connecting the absorption apparatus. 5 ml. of 0.25 N sodium hydroxide added to the absorption tube. The absorption apparatus was connected, the rate of flow of nitrogen adjusted to about one bubble every 2-3 seconds. It was then heated on an oil bath at 145°C for 2 hours. The bath was then removed and the flow rate of nitrogen increased to 2-3 bubbles per second for 10 minutes. The absorption apparatus was then disconnectd and the contents carefulley transferred to and washed (5×5 ml) in a conical flask.

2 ml of barium chloride (10%) and two drops of phenolphthalein were added to the titration flask and the excess alkali was titrated against standard 0.1 N hydrochloric acid. Blanks were also performed.

Found uronide content 33.42%.

Hydrolysis of the mucilage and identification of the sugars in the hydrolysate :

0.05 gm of the mucilage was refluxed with 5ml of 2 N sulphuric acid on a water bath for 18 hours. The filtrate was neutralised with barium carbonate, filtered and concentrated under reduced pressure.

On paper chromatography in butanol: ethanol: water : ammonia (45: 5: 49: 1), the concentrated syrup gave two spots, the Rf values of these were measured and compared with those given in literature[5] for various sugars.

Rf. found	*Rf given*	*Sugar identified*
0.048	—	oligosaccharide
0.18	0.17	xylose.

Mucilage also hydrolysed by N sulphuric acid (30 hrs). The same two sugars were obtained.

Hydrolysis with formic acid :

A sample of mucilage was hydrolysed by being dissolved in formic acid (98-100%) and the solution heated in a sealed tube immersed in a boiling water bath for seven hours. .The tube was then cooled, the contents poured into an equal volume of water and the solution evaporated to dryness. The residue was suspended in N sulphuric acid and the mixture heated on a boiling water bath for six hours. Acid was removed from the cooled solution with De-acidite E and the filtered solution then evaporated to a syrup. Examination of the syrup on the chromatogram showed the presence of xylose and an oligosaccharide.

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SEMIMICRO DETERMINATION OF SAPONIFICATION EQUIVALENT OF HIGHER FATTY ACID ESTERS

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SUMMARY

Micro and Semimicro determination of neutralization equivalents of higher fatty acids has been reported earlier by the authors. The method has been further extended for the semimicro determination of Saponification equivalents of methyl esters of higher fatty acids. It has been observed that while dealing with fats and fat products, some times one comes across with smaller quantities of the products and in such a case the analysis of the product becomes difficult with usual macromethods.

20-30 mg sample was accurately weighed in a pyrex glass vial and transferred to a flask which had arrangement for passing Nitrogen gas. An excess of ethanolic potassium hydroxide (10ml of 0.02 N) was added and heated for 1/2hr under reflux, passing nitrogen gas all the time through the flask. After 1/2hr the contents were cooled and the excess alkali back titrated against standard solution of methanolic hydrogen chloride using 3-4 drops of the mixed indicator (A mixture of 0.4% methanolic solutions of phenol red, cresol red and Bromothymol blue in the ratio of 3 : 1 : 1).

A blank determination was simultaneously carried out under identical conditions.

Determination carried out with the esters of lauric, myristic and palmitic acids showed that the results were accurate to within $\pm 1\%$ of the theoretical values.

Number of simple volumetric procedures for the determination of highe; fatty acid esters in the semimicro and microscales is not large in the literaturer though some other good methods like colorimetric method of Hill[1,2] and few recently reported chromatographic methods[3,4] are available for the purpose. However, these methods, though accurate, are not always convenient, as they require specific instruments and are not rapid. The necessity of a semi-micro volumetric method for the determination of higher fatty acid esters arose out of the fact that quite often, the quantities of fatty products of plant or animal origin are available in small amounts for investigations. Further, sometimes fractions of esters of fatty acids of animal origin viz. that of the fish Lebeorohita have colour which deepens in the presence of alkali, with the result that phenolphthalein indicator does not work satisfactorily.

In a previous communication[5] by the authors, a semimicro procedure for the determination of neutralization equivalents of higher fatty acids in non-aqueous solvents has been reported, using a mixed indicator of phenol red, cresol red and bromothymol blue. The use of this mixed indicator is further described here and a simple, rapid and accurate semimicro procedure for the determination of saponification epuivalents of higher fatty acid esters, is reported.

In a few preliminary experiments potassium methoxide was used as the saponifying agent, but no appreciable amount of reaction took place. The use of alcoholic potassium hydroxide gave good results. After the saponification, excess alkali was back titrated against standard solution of methanolic hydrogen chloride using 3-4 drops of the mixed indicator. A blank was simultaneously run under identical conditions. Nitrogen gas was passed through the solution during the entire procedure.

EXPERIMENTAL

Reagents and Materials :

(i) A 0.02N alcoholic potassium hydroxide solution was made by dissolving A. R. grade potassium hydroxide in aldehyde-free ethanol. The solution was deoxygenated by passing nitrogen gas through it and stored in a glass stoppered flask. The solution was discarded as soon as it was even slightly discoloured.

(ii) *0.02N methanolic hydrogen chloride solution.*—Hydrogen chloride gas was passed in A. R. grade methanol, till it was saturated. The solution was poured in a beaker and allowed to stand overnight. Its approximate strength was determined by titration against alkali and then diluted to the requisite strength by methanol and standardised, using 3-4 drops of the mixed indicator described below.

(iii) *Indicator.*—0.4% methanolic solutions of phenol red, cresol red and bromothymol blue. A mixture of the three in the ratio of 3 : 1 : 1 by volume was prepared. The indicators should be prepared afresh each day and stored in coloured glass bottles.

(iv) A pyrex glass-150 ml flask-with an arrangement for passing nitrogen gas and a B_{14} joint at the top, a magnetic stirrer and a day light lamp.

Procedure :

20-30 mg sample was accurately weighed by a semimicro balance in a pyrex glass vial and transferred to the reaction flask. The flask was attached to the nitrogen supply and the gas passed for about five minutes. 10.0ml of ethanolic potossium hydroxide were added ; the flask shaken a little and the contents refluxed on a water bath by attaching an air condenser to the flask. After an initial heating of about 10 minutes, when the contents began to boil, the heating was continued for further 30 minutes. The flask was removed from the water bath, cooled and the excess alkali back titrated against standard solution of methanolic hydrogen chloride, using 3-4 drops of the mixed indicator. The colour change at the end point was from pink violet to yellow. The solution was stirred by a magnetic stirrer during the course of titration and the end point viewed by using a day light lamp.

A blank was run simultaneously under the identical conditions.

The results of the determinations on the methyl esters of lauric, myristic and palmitic acids given in the table show an accuracy of within $\pm$ 1% of the theoretical.

TABLE

Saponification Equivalents of some higher fatty acid esters.

Methyl Ester of	Sap. Equivalent	
	Cal.	found
Lauric	214.00	213.10, 212.46
Myristic	242.00	241.36, 243.89
Palmitic	270.00	267.67, 268.34

ACKNOWLEDGEMENT

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CHEMICAL EXAMINATION OF THE COLOURED CONSTITUENTS FROM THE SEEDS OF CASSIA ALATA

By

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SUMMARY

Three different coloured compounds

(*i*) $C_{17}H_{14}O_7$, orange red, m.p. 212°C,

(*ii*) $C_{15}H_{10}O_4$, dark yellow, m.p. 194°C, and

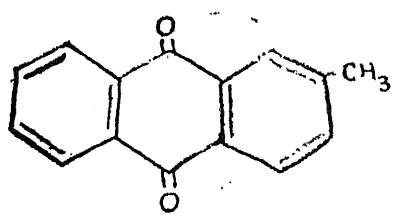
(*iii*) $C_{13}H_8O_4$, yellow, m.p. 164°C, have been isolated from the seeds of Cassia alata. The orange red compound $C_{17}H_{14}O_7$ was found to be a dihydroxy, dimethoxy derivative of β-methyl anthracene. The relative positions of the groups are under investigation. The yellow compound $C_{15}H_{10}O_4$, has been found to be identical with chrysophanic acid (4, 5-dihydroxy-2-methyl anthraquinone). The yellow compound $C_{13}H_8O_4$, does not seem to be related to anthraquinone structure. It is a dihydroxy xanthone. From the melting point of different dihydroxy derivatives and their derivatives given in literature, it appears to be 1:2 dihydroxy xanthone.

Cassia alata commonly known as Dadrughna or Dadmurdan, is quite a well known plant belonging to the Leguminosae family. The various parts of the plant have been described to be highly medicinal[6].

As a result of extraction of the powdered seeds of Cassia alata with hot carbon-tetrachloride followed by subsequent purification by repeated fractional crystallisation and chromatography, the following three compounds have been isolated in the pure form:

1. Orange red (A), m.p. 212°C, $C_{17}H_{14}O_6$
2. Dark yellow (B), m.p. 194°C, $C_{15}H_{10}O_4$
3. Bright yellow (C), m.p. 164°C, $C_{13}H_8O_4$

The orange red coloured compound (A), m.p. 212°C and molecular formula $C_{17}H_{14}O_6$ on analysis was found to contain two methoxyl groups, two phenolic hydroxyl groups and on zinc dust distillation gave β-methylanthracene. The reaction with 2:4-dinitrophenyl hydrazine and other reagents together with a peak at 1683 cm^{-1}, show that it is related to anthraquinone. All the six oxygen atoms of the compound are thus accounted for, two as phenolic hydroxyl, two as methoxyl and two as quinone. Since β-methyl anthracene is obtained as a result of deoxygenation of the compound, it is clear that this is the basic parent skeleton in its structure which can be written as

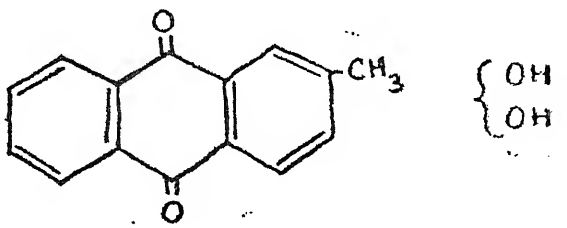


{2OH
{2OCH3

The relative positions of these groups are under investigation.

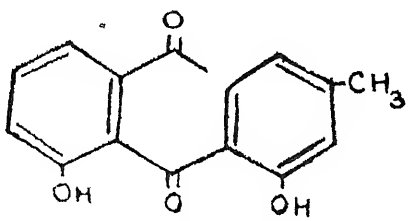
The dark yellow coloured compound (B), m.p. 194°C, on the basis of its analysis and molecular weight determination was found to be $C_{15}H_{10}O_4$. It formed a diacetate showing the presence of two hydroxyl groups. On zinc dust distillation it gave β-methylanthracene showing thereby that this must be the basic structural unit in the compound. The colour reactions and its characteristic absorption in the infra-red spectrum definitely show the presence of a quinone structure and from the nature of the reactions it appears that at least one of the hydroxyl groups must be in the α-position with respect to the carbonyl group.

Thus the compound appears to have the following structure:



From the study of the various di-oxy-2-methyl-anthraquinones described in literature the comound under examination appears to resemble chrysophanic acid, m.p. 196°C, which is 4:5-dihydroxy-2-methyl-anthraquinone. This was confirmed by determination of mixed melting point with an authentic sample of chrysophanic acid when no depression in the melting point was observed. The melting point of the diacetate also resembles the melting point of the diacetate of chrysophanic acid.

Thus the compound (B) is identical with chrysophanic acid which is 4,5-dihydroxy-2-methyl-anthraquinone having the following structure:

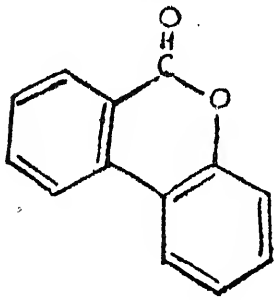


The occurrence of chroysophanic acid in the plants of Cassia species is not an isolated observation. It is known to occur in the wood of Cassia siamea[4], in Goa or Araroba powder[10], in the heart wood of Ferreira spectabilis[5], in the seeds of Cassia obtusifolia[9] and in small amouts in five common samples of senna leaves[1].

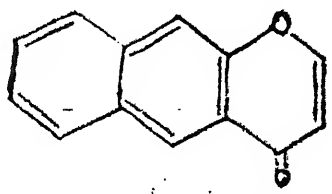
The bright yellow coloured compound (C) m.p. 164°C, molecular formula $C_{13}H_8O_4$, formed a diacetate $C_{17}H_{18}O_6$, m.p. 188°C, showing the presence of two hydroxyl groups. When methylated using dimethyl sulphate and potassium hydroxide, it formed a compound $C_{15}H_{12}O_4$, m.p. 129°C which on analysis was found to contain two methoxyl groups whereas the original compound $C_{13}H_8O_4$ did not give any methoxyl value, which further confirms the presence of two phenolic hydroxyl groups.

Unlike the compounds (A) and (B) this does not seem to be related to anthraquinone structure as neither any colour reactions nor any characteristic peaks in the infra-red spectrum in the region of 1685 cm^{-1}, indicating the presence of a quin-

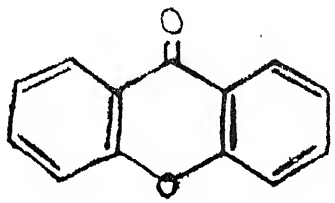
one structure were obtained. The compound does not react with hydroxylamine, phenylhydrazine or semi-carbazide. There is, however, a pronounced peak at 1763 cm^{-1} which is not present in either the compound (A) or (B) and this peak is usually characteristic of compounds containing a sort of modified $>C=O$ structural unit as present in the case of lactones or pyrones showing that structural unit of one of the following types together with 2-phenolic hydroxyl groups in some positions may be present in this compound:



LACTONE

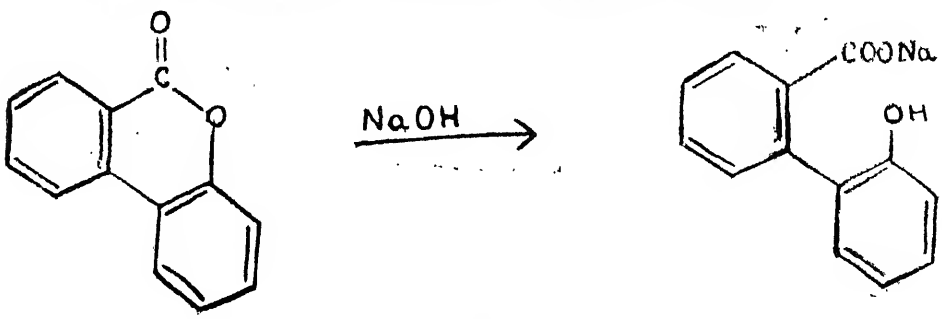


NAPHTHOPYRONE



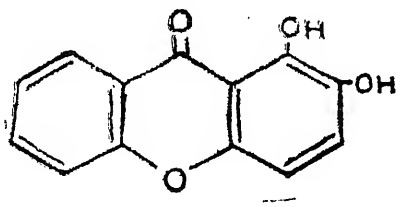
DIBENZOPYRONE
(XANTHONE)

Out of these three possibilities, the first one, namely the compound being a lactone can easily be eliminated because the completely methylated product of (C), is insoluble in alkali whereas if the compound had a lactone structure, not only the original compound but also the methylated product would be soluble in alkali owing to the opening of the lactone ring in presence of alkali.



The presence of a naphthopyrone derivative in the seeds of Cassia tora has been reported recently by S. Rangaswami[7], but the infra-red spectrum of the compound is quite different from the infra-red spectrum of the compoud isolated from the seeds of Cassia alata. Hence the compound (C) appears to have a xanthone structure with two phenolic hydroxyl groups, i.e., it is a dihydroxy xanthone.

From the melting points of the different dihydroxy xanthones given in literature, it appears to be 1:2 dihydroxy xanthone.



This is suported by the melting points of the dimethyl ether and the diacetate.

EXPERIMENTAL

The material employed for these investigations consisted of the authentic seeds of Cassia alata obtained from the Punjab Ayurvedic Pharmacy, Amritsar.

The crushed material was extracted with carbon-tetrachloride. The extract obtained as a result of the first and the second extractions was dark orange in colour and when the solvent was distilled off an oil was obtained which when allowed to stand deposited a dark orange-red coloured residue. When more of the carbon-tetrachloride was added to the material which had been twice extracted as above and refluxed, the colour of the extract this time was yellowish-red and on distilling off the solvent, an oil was obtained which deposited reddish-yellow residue. Further extraction with carbon-tetrachloride under the same conditions and removal of the solvent furnished a product which was yellow in colour.

The orange-red product obtained during the first two extractions was crystallised from toluene and as a result of repeated crystallisations an orange-red compound (A), m.p. 212°C was obtained. The melting point of the product did not rise on further crystallisations. It was further purified by chromatography over cellulose and eluting with toluene.

The product obtained during the third and the fourth extractions which was yellowish red in colour, was purified by fractional crystallisation from a mixture of alcohol and benzene 1:1 when the two compounds (C) bright yellow, m.p. 164°C and the other (B) dark yellow, m.p. 194°C were obtained.

These two products were also obtained by fractional crystallisation of the residues obtained during the fifth and subsequent extractions. Both these compounds were further purified by repeated crystallisation followed by chromatography over cellulose column.

Examination of the compound (A):

The compound (A), m.p. 212°C was readily soluble in chloroform, moderately soluble in glacial acetic acid and less soluble in alcohol. It dissolved in concentra-

ted alkali giving bluish-violet solution from which the original compound got precipitated on acidification with mineral acids. In ethanolic solution it gave a brownish colour with ethanolic ferric chloride. The compound did not give any effervescence with sodium bicarbonate but a positive test with 2:4 dinitrophenyl hydrazine was obtained.

Analysis

Found	*Calculated for* $C_{17}H_{14}O_6$
C=64.63°/o	C=65.0°/o
H=5.80%	H= 4.5°/o
Mol. wt. =322 (Rast)	Mol. wt.= 314

Determination of alkoxyl group:

The determination of alkoxyl group in the compound was done by the method of Belcher, Fildes and Nutten[2].

Found methoxyl percentage in (A) =17.9°/o

Calculated for two methoxyl groups =19.7°/o in $C_{17}H_{14}O_6$.

Acetylation of the compound (A):

The compound (A) was acetylated with acetic anhydride and sodium acetate. The crude acetyl derivative was crystallised from absolute ethanol. The melting point of the acetyl derivative was found to be 198°C.

The percentage of acetyl groups in the acetylated product was determined by the method of Wiesenberger as described by Belcher and Godbert[3a].

Found	*Calculated for* $C_{17}H_{12}O_4(O.CO.CH_3)_2$
C=63.03°/o	C=63.31°/o
H= 4.90°/o	H= 4.52°/o
Mol. wt. =383	Mol. wt. =398
Acetyl percentage=21.3°/o	Acetyl percentage =21.6%

Preparation of oxime of the compound (A):

The oxime of the compound (A) was prepared by following the method of Shriner, Fuson and Curtin[8]. The oxime was crystallised from ethanol when a compound m.p. 198°C, was obtained.

Nitrogen was estimated in the oxime by Dumas method[3b].

Found	*Calculated for* $C_{17}H_{16}O_6N_2$ (2C=O gr.)
Nitrogen in oxime=8.81%	Nitrogen in oxime=8.10%

Examination of the compound (B):

The dark yellow compound (B), m.p. 194°C, was soluble in ethanol and benzene. It dissolved in alkali giving a red solution from which the original compound got precipitated on acidification with mineral acids. In ethanolic solution, it gave a dark brown red colour with ethanolic ferric chloride. It did not react with sodium bicarbonate but a positive test was obtained with 2:4 dinitrophenyl hydrazine reagent.

Analysis

Found	*Calculated for* $C_{15}H_{10}O_4$
C=70.53%	C=70.87%
H= 4.27%	H= 3.94%
Mol. wt. =269 (Rast)	Mol. wt. =254

Acetylation of the compound (B):

The compound (B) was acetylated by treating with fused sodium acetate and acetic anhyride. The crude product was crystallised from benzene when a probuct m.p. 208°C was obtained.

The percentage of acetyl group was determined by the method of Wiesenberger[11,3a].

Found	*Calculated for* $C_{15}H_8O_2(O.CO.CH_3)_2$
C=67.14%	C=67.45%
H= 4.39%	H= 4.14%
Mol. wt. =346 (Rast)	Mol.wt. =338
Acetyl percentage=24.10%	Acetyl percentage=25.45%

Methylation of the compound (B):

The compound was methylated with dimethyl sulphate and potassium hydroxide. The crude product was crystallised from dilute alcohol as yellow orange needles, m.p. 195°C.

Percentage of the methoxyl groups was determined by the method of Belcher, Fildes and Nutten[2].

Found	*Calculated for* $C_{17}H_{14}O_4$
Methoxyl percentage = 20.83%	Methoxyl percentage = 21.99%

Examination of the Compound (C):

The bright yellow coloured compound (C) m.p. 164°C, was soluble in ethanol, benzene and toluene. It did not react with hydroxylamine or phenyl hydrazine. Its solution in ethanol gave a green colour with ethanolic ferric chloride.

Analysis:

Found	*Calculated for* $C_{13}H_8O_4$
C = 68.10%	C = 68.4%
H = 3.91%	H = 3.5%
Mol. wt. = 240 (Rast)	Mol. wt. = 228

Acetylation of the compound (C):

The compound was acetylated by treating it with fused sodium acetate and acetic anhydride. The crude product was crystallised from absolute ethanol and was found to melt at 183°C.

Found	*Calculated for* $C_{17}H_{12}O_6$
C = 64.98%	C = 65.37%
H = 4.32%	H = 3.85%
Acetyl group = 26.13%	Acetyl group = 27.56%

Methylation of the Compound (C):

The compound (C) was methylated with potassium hydroxide and dimethyl sulphate. The product thus obtained was crystallised from ethanol. The melting point of the methylated product was found to be 129°C.

The methoxyl percentage in the compound was determined by the method of Belcher, Fildes and Nutten[2].

Found	*Calculated for* $C_{15}H_{12}O_4$
Methoxyl percentage = 22.67%	Methoxyl percentage = 24.22%

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THE FATTY ACID COMPOSITION OF THE FERONIA ELEPHANTUM TRUNK BARK FAT

By

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SUMMARY

The bark fat of Feronia elephantum contains palmitic, stearic oleic and linoleic acids as 3.71, 5.42, 87.13 and 3.74% respectively. The composition of acids in general agrees with other bark fats in small amounts of palmitic and stearic acids and in large amounts of oleic acid. The nonsaponifiable matter of the fat consists of two ingredients, β-sitosterol to the extent of 21% of the fat and an unsaturated aliphatic liquid which could not be identified. On account of the high percentage of β-sitosterol, it appears that the fatty acids besides being present in the form of glycerides are also present in combination with β-sitosterol.

The bark fat of Feronia elephantum, when allowed to stand in concentrated petroleum ether solution overnight in a frigidaire, gave a greenish yellow deposit which, after purification by chromatography over alumina followed by subsequent crystallisation of the product obtained after elution, was found to be a sterol. It melted at 136-137°C, gave all the colour reactions of phytosterol and analysed as $C_{29}H_{50}O$. A comparison of the melting point, analysis, infra-red spectrum and melting points of acetate, benzoate and digitonide clearly suggest that the compound is identical with β-sitosterol. This has further been confirmed by the determination of mixed melting point with an authentic sample of β-sitosterol.

The fat left after the separation of free β-sitosterol was saponified and as a result of the study of its component acids by ester fractionation it was found to contain palmitic acid 3.71%, stearic acid 5.42%, oleic acid 87.13% and linoleic acid 3.74%.

This composition of the fatty acids is in agreement with the observation that the bark fats usually consist of oleic acid as the major component along with smaller amounts of palmitic and stearic acids.[1]

The ether extract of the fat-soap showed the presence of more of β-sitosterol along with a very small amount of a liquid unsaturated in character which could not be identified. Since this constitutes about 21% of the fat it appears that the fatty acids besides being present in the form of glycerol esters are also present in combination with β-sitosterol.

EXPERIMENTAL

The crude mixture of water insoluble compounds obtained when the concentrated ethanolic extract of the trunk bark of Feronia elephantum was poured in a large excess of water, was exhaustively extracted with petroleum ether till a colourless extract was obtained. All the subsequent extracts were combined and concentrated to a small volume by distillation under reduced pressure on a water bath. The concentrated extract was allowed to stand overnight in a refrigerator when a slightly yellowish green product separated out. The product was filtered, washed with chilled petroleum ether, dissolved in ether and chromatographed over Brockmann's alumina. After elution with petroleum ether-benzene mixture (9 : 1) and evaporation of the solvent an almost colourless solid was obtained which when crystallised from methanol yielded colourless plates, melting point 135-136°C.

The compound was soluble in cold benzene, chloroform, ether, petroleum ether and in hot ethanol and methanol but insoluble in water. It was optically active and neither did it reacted with 2 : 4-dinitrophenylhydrazine reagent, nor reduced Fehling's solution.

A violet colour changing to blue on adding 20 drops of acetic anhydride and 2 drops of concentrated sulphuric acid to a 2 ml solution of compound in chloroform, a red colour on adding concentrated sulphuric acid to the compound in chloroform and a dark red colour changing to yellow on dilution on adding concentrated sulphuric acid to the compound in acetic anhydride were observed. A reddish violet colour on adding trichloroacetic acid and water (9 : 1) to the compound and a violet colour on adding concentrated sulphuric acid and water (5 : 1) to the compound were observed. A white precipitate was obtained when ethanolic solutions of the compound and digitonin were mixed.

Analysis

Found	Calculated for $C_{29}H_{50}O$
C = 84.20%	C=84.05%
H = 11.89%	H=12.07%
Mol. wt = 420 (cryoscopic in benzene)	Mol. wt.=414

Rotation $[\alpha]_D^{25} = -37°$ (Ca. 1.5 in chloroform).

Infra-red spectrum.

The peaks 3497cm^{-1} (2.86mμ,—OH)[2] 2899cm^{-1} (3.45mμ,—CH_3,—CH_2—)[3] ; 1639cm^{-1} (6.1 mμ, C=C stretching in steroids)[4,5] were obtained in the I R, spectrum of the compound.

Acetylation :

0.2 g of the compound were acetylated in the usual way. The acetylated product after crystallisation from methanol was found to melt at 126-127°C.

Benzoylation :

0.1 g of the compound were benzoylated using benzoyl chloride and dry pyridine. The benzoyl derivative after crystallisation from methanol melted at 143-144°C.

Digitonide :

0.1 g of the sterol were treated with 0.1g of digitonin in ethanol. The digitonide after crystallisation from ethanol melted at 221°C (decomp.).

With the help of the above properties and determination of mixed melting point with an authentic sample, the compound was identified as β-sitosterol.

Petroleum ether soluble part :

The petroleum ether soluble part left after the separation of β-sitosterol was purified by boiling with a pinch of animal charcoal and when the solvent was distilled off a dark green bark fat was obtained.

The fat on analysis was found to have

Saponification value = 193.5
Iodine value = 80.3

The fat was saponified with 0.5N ethanolic potassium hydroxide solution over a water bath, ethanol distilled off, the soap dissolved in water and extracted thrice with 150 ml of ether each time. The ethereal extract was washed free of alkali, dried over fused sodium sulphate and the solvent evaporated when an orange coloured viscous substance was obtained in 24.2% yield.

Fatty acids :

The aqueous alkaline soap solution was decomposed by the addition of mineral acid and the liberated fatty acids were taken up in ether. The ethereal solution was washed free of mineral acid, dried over fused sodium sulphate and the solvent evaporated when fatty acids were obtained which on analysis gave the following constants :

Mean molecular weight = 281.1
Iodine value = 86.1

As only a small quantity of these acids were available they were directly converted into their methyl esters which were fractionated under reduced pressure when four different fractions were obtained. The weights of the different fractions were taken and saponification and iodine values of each were determined. The composition of each fraction was calculated with the help of these values. The observations and the results are reported in table 1.

TABLE No. 1

Fractionation of the methyl esters of mixed fatty acids and the calculated composition of the ester fractions

S. No.	Wt.	H. T.	Pressure	S. V.	S. E.	IV.	Sat.	Unsat.	Pal	St	Ol	Li
1	3·53	148—152	1·5mm	197·7	283·2	80·4	0·36	3·17	0·18	0·18	3·03	0·14
2	2·13	152—154	,,	196·3	285·4	83·2	0·14	1·99	0·06	0·08	1·87	0·12
3	1·97	154—156	,,	197·0	284·3	83·6	0·13	1·84	0·06	0·07	1·76	0·08
4	2·51	Above 198°C	,,	193·1	290·20	77·0	0·30	2·21	0·08	0·22	2·17	0·04
	10·14				Total		0·93	9·21	0·38	0·55	8·83	0·38
					% esters				3·74	5·42	87·10	3·74
					% acids				3·71	5·42	87·13	3·74
					% mole				4·05	5·37	86·83	3·75

Pal = Palmitic ; St = Stearic ; Ol = Oleic and Li = Linoleic

Examination of the orange coloured viscous liquid :

The orange viscous liquid obtained as described earlier, was subjected to fractional crystallisation using methanol when two different products A and B were separated. Product A, on subsequent purification by crystallisation and column chromatography over Brockmann's alumina, gave a compound melting at 136-137°C and was identified to be β-sitosterol by the preparation of a few derivatives and some colour reactions described earlier. Product B which was a viscous liquid at ordinary temperature was purified by fractional distillation under vacuum at 239°/9.5mm. The pure product so obtained was an orange coloured viscous liquid in very small amount.

It was unsaturated and aliphatic in nature, however, it could not be identified.

ACKNOWLEDGEMENT

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ISOLATION AND STUDY OF THE MUCILAGE FROM THE SEEDS OF DIPTERACANTHUS PROSTRATUS NEES

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ABSTRACT

The defatted seeds of Dipteracanthus prostratus Nees were extracted with acidulated water, precipitated with ethanol and this procedure repeated for purification. On preliminary analysis the mucilage was found to have methoxyl 5.22% and acetyl 3.51%. The mucilage was hydrolysed with 2N sulphuric acid. Qualitative and quantitative analysis of the hydrolysate revealed galactose 21.2%; arabinose 15.7%; fucose 19.1% and galacturonic acid 22.14%. Methylation with dimethyl sulphate and alkali gave a methylated product which on hydrolysis and chromatographic examination gave 2,3,4-tri-O-methyl-D-galacturonic acid; 2-O-methyl-L-fucose; 2,3-di-O-methyl-L-arabinose; 2,3,6-tri O-methyl-D-galactose and 2,3,4,6-tetra-O-methyl-D-galactose. On the basis of these a structure has been assigned to the mucilage.

Dipteracanthus prostratus Nees or Ruellia prostrata Poir belonging to the natural order Acanthaceae grows throughout India. The leaves have medicinal properties[1,2].

The mucilage has been isolated by extracting the crushed defatted seeds with water and precipitating with ethanol. This procedure has been repeated several times till a pure product is obtained.

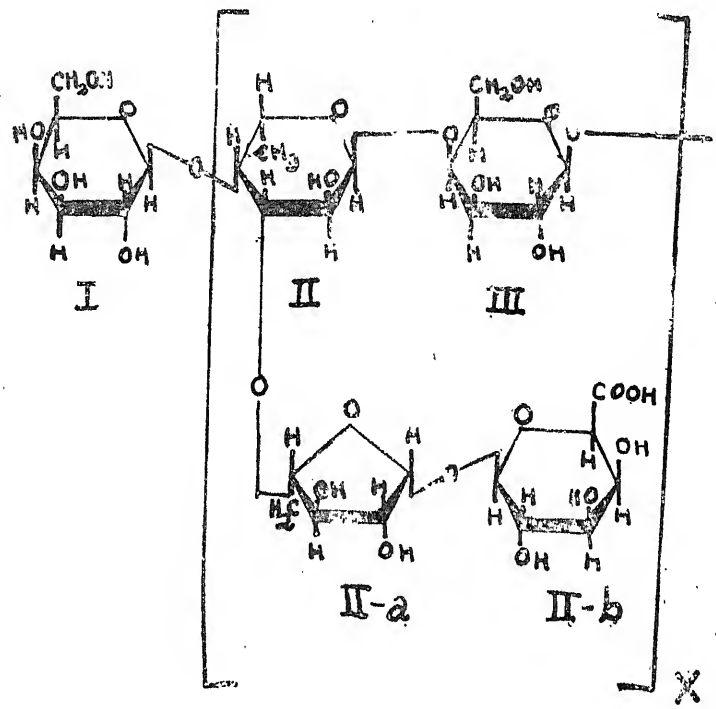
On preliminary analysis the mucilage gave methoxyl 5.22%; acetyl 3.51% and uronide 22.14%. On hydrolysis with 2N sulphuric acid and paper chromatographic examination of the hydrolysate the presence of D-galactose, L-arabinose, L-fucose and D-galacturonic acid was revealed. These sugars were determined quantitatively by elution of each sugar from the chromatogram and periodate oxidation of each elute and were found to be galactose 21.2%; arabinose 15.7% and fucose 19.1%.

Methylation of the mucilage was carried out with dimethyl sulphate and alkali[3] giving a methylated product $(\alpha)_D^{30}$ + 132.06° (in chloroform) which was hydrolysed with 1N sulphuric acid and methanol. The hydrolysate after neutralisation and concentration was examined on paper chromatograms. 2,3,4-Tri-O-methyl-D-galacturonic acid; 2-O-methyl-L-fucose; 2,3,-di-O-methyl-L-arabinose; 2,3,6-tri-O-methyl-D-galactose and 2,3,4,6-tetra-O-methyl-D-galactose were found to be present in the hydrolysate.

Since 2,3,4,6-tetra-O-methyl-D-galactopyranose and 2,3,4-tri-O-methyl-D-galactopyranosyluronic acid have been found in the hydrolysate of the methylated mucilage, it is evident that each of these are linked only through their C_1 and consequently form terminal groups. In view of their relative proportions however the terminal end must be 2,3,4,6-tetra-O-methyl-D-galactose and the methylated uronic acid must be linked as shown by II-b in the structure. 2-O-methyl-L-fucose (II) has positions C_1, C_3 and C_4 free hence forms the branching point in the mucilage. The presence of 2,3,6-tri-O-methyl-D-galactose indicates that this molecule (III) is linked through its C_1 and C_4 positions to II and the repeating unit. 2,3-Di-O-methyl-L-arabinose (II-a) forms the side chain linked to the fucose molecule by its C_5 and to the uronic acid by C_1. The relative proportions of the sugars and sugar acids are in fairly good agreement with those determined quantitatively. It is thus evident that fucose and galactose form the main chain and arabinose and galacturonic acid together constitute the side chain

of the aldobiuronic acid 1-O-(α-D-galactopyranosyluronic acid)-L-arabinose which is linked to the fucose molecule II.

STRUCTURE



EXPERIMENTAL

Unless otherwise stated, paper chromatographic separations were carried out on Whatman No. 1 paper using the following solvents (A) butanol : ethanol : water (40:10:50)[4] (B) butanol : ethanol : ammonia : water (40:10:1:49) (C) butanol : acetic acid : water (5:1:4). The spray reagents used were aniline hydrogen phthalate, silver nitrate in acetone and ethanolic sodium hydroxide; naphthoresorcinol and trichloracetic acid.

The seeds were powdered, washed with water, dried and extracted exhaustively with petroleum ether (40°-60°), acetone and ether. The defatted seeds were suspended in water acidified with acetic acid and stirred mechanically for 36 hours. Each batch of seeds was thus extracted three to four times so as to dissolve the maximum amount of mucilage. The extract was filtered and added slowly with stirring to 90% ethanol. The crude precipitated mucilage was filtered, washed with ethanol and ether and dried in vacuum. This procedure was repeated several times till the product gave negative test for nitrogen and the ash content was reduced to a minimum.

Methoxyl group :—The methoxyl group was determined by heating the mucilage with hydriodic acid and estimating the methyl iodide by the method of Belcher et al[5]. Found methoxyl 5.22%.

Acetyl ester was determined by saponification with sulphuric acid according to the method of Wiesenberger[6] as described by Belcher[7]. Found acetyl ester 3.51%.

Uronide content :—0.025 gm of mucilage was hydrolysed with 19% M. A. R. hydrochloric acid. The carbon dioxide formed was absorbed in 5% sodium hydroxide and estimated volumetrically in accordance to the method of Barker, Foster, Siddiqui and Stacey[8]. Found uronide content 22.14%.

Hydrolysis and identification of the sugars in the hydrolysate :—0.05 Gm of mucilage was hydrolysed with 2N sulphuric acid for 72 hrs. The hydrolysate was neutralised with barium carbonate, filtered and concentrated under reduced pressure. Paper chromatograms of the hydrolysate were run in solvents (B) and (C) and each sprayed with a different spray reagent. The Rf values were measured and compared with those given in literature[4,9].

Solvent (B)		*Solvent (C)*		
Rf found	Rf given	Rf found	Rf given	Sugar identified
0.085	0.09	0.15	0.16	D-galactose
0.135	0.145	0.20	0.21	L-arabinose
0.16	...	0.28	0.27	L-fucose
0.024	...	0.13	0.14	D-galacturonic acid

Another chromatogram with spots of the hydrolysate and reference sugars galactose, arabinose, fucose and galacturonic acid was run in solvent (A). The spots of the hydrolysate sugars corresponded with those of the reference sugars.

Quantitative estimation of the monosaccharides in the hydrolysate. 0.5 Gm of purified mucilage was refluxed with 1.5 N sulphuric acid and neutralised with barium carbonate. The filtrate was made up to 25 ml and a known volume applied on a paper chromatogram along with guide spots. After developing in solvent (B), the strips corresponding to the guide spots were cut and eluted. The elutes were oxidised with periodate and the quantity of monosaccharides determined. Blanks were run simultaneously.

Found :—Arabinose 15.7%; Galactose 21.2%, Fucose 19.1%.

Methylation :—The mucilage (3 gms) was dissolved in water (20 ml), dimethyl sulphate (30 ml), and 40% sodium hydroxide (60 ml), were added with constant stirring during 8 hrs. The temperature was maintained at 30° during addition. The mixture was remethylated and the thick syrup concentrated and dissolved in acetone. The acetone solution was remethylated thrice and the concentrated syrup extracted exhaustively with chloroform in a liquid-liquid extractor (downward displacement type) for 20 hrs. The chloroform extract was dried over anhydrous sodium sulphate and distilled under reduced pressure.

The methylated product $(\alpha)_D^{30}$ ^{30}+132.06° in $CHCl_3$ was hydrolysed with 1N sulphuric acid containing methanol, at 100° for 24 hours.

After removing sulphuric acid with barium carbonate, the hydrolysate was concentrated and spots applied on Whatman No. 1 paper. The chromatogram was run in Solvent (A). The Rg values (distance travelled by methylated sugar divided by distance moved by 2,3,4,6-tetra-O-methyl-D-glucose) of the methylated sugars were determined and compared with those given in literature[10].

The following methylated sugars were identified.

Rg found	*Rg given*	*Sugar identified.*
0.25	...	2, 3, 4-tri-O-methyl-D-galacturonic acid
0.52	0.51	2-O-methyl-L-fucose
0.62	0.61	2, 3,-di-O-methyl-L-arabinose
0.72	0.71	2, 3, 6-tri-O-methyl-D-galactose
0.87	0.88	2, 3, 4, 6-tetra-O-methyl-D-galactose

As the Rg values of methylated uronic acids were not mentioned in literature the identification of these were carried out by a different method[11].

The barium salt of the uronic acid was prepared by treating the hydrolysate of the methylated mucilage with barium hydroxide at 100° for 5 hr. The barium salt thus formed was isolated by concentration of the aqueous solution and the barium ions removed. The free acid was reduced by lithium aluminium hydride. The uronic acid after reduction was examined paper chromatographically. One spot Rg 0.63 was revealed on the chromatogram. This corresponded to 2,3,4-tri-O-methyl-D-galactose (given Rg. 0.64)[12]. Thus the methylated uronic acid was identified as 2,3,4-tri-O-methyl-D-galacturonic acid.

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APPLICATION OF MASS SPECTROMETRY IN THE STRUCTURAL DETERMINATION OF ALKALOIDS

By

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As far back as in 1921, Sir J. J. Thomson[1] could foresee that " . . . . there is no branch of science where promise of great discoveries is more hopeful than those which will result by researches which involve the application of physical principles and physical measurements to chemical phenomenon." Thirty-five years later, in 1956, Professor Woodward[2] observed, "Physical methods and the principle that they should be used whenever possible are now part of our armamentarium . . . .nuclear magnetic resonance is even now on the horizon, and we shall be surprised if it does not permit another great step forward." Indeed this prediction came true. But perhaps, the most dramatic development in the application of physical methods to structural problems in organic chemistry has been with the mass spectrometry. We owe a great deal to the pioneering work of Professor Biemann[3] and to the zeal of Professor Carl Djerassi[4] in developing this tool with remarkable rapidity and precision.

Unlike the other physical methods, mass spectrometry does not directly indicate the presence or absence of functional groups but it rather characterizes the molecular species of the sample. In a way, it is a sort of degration unknown to the classical chemists yet it follows certain rational patterns which could be verified by the presence of appropriate ion peaks. The chief advantages, *viz.* (i) determination of exact molecular weight leading to the most possible empirical formula which analysis may fail to achieve, (ii) complementary nature of ultraviolet and mass spectrometry and above all (iii) requirement of even a less than a milligramme of material to have the greatest possible structural information in the shortest possible time, make this method indispensable atleast to the plant chemists. Purity of a specimen can often be judged from the mass spectrum. For instance, the spectrum of ibogaine (methoxy ibogamine, mol. wt. 310) showed an intense peak at 280 corresponding to the molecular ion of the associated ibogamine which can be removed from the former only with difficulty[5]. It is, of course, sometimes necessary to supplement information by other physical or chemical methods though examples are accumulating (*viz.* Ibogaline[5], pyrifoline, refractidine[6] lochneridine[7] and our own work[8] on glycorine, glycosmicine and glycosminine) where the correct structures have been elucidated without recourse to classical degradation of any type. Thus, though still in its infancy, this method of immense potentiality is going to stay with the organic chemists.

The principle and practice involved in mass spectrometry have been exhaustively dealt with by Beynon[9] and Biemann[10]. The principal application in the structure elucidation of plant products has been in the field of alkaloids we are concerned with besides steroids[4] and triterpenoids[4a]. In the field of alkaloids, this method has been applied to the indole[11] dihydroindole[4,12] and α-methyleneindolenine[13, 14], quinoline and isoquinoline[15,16], quinazoline[8], pyrrolizidine[17], tropane[18], aporphine[19] and colchicine[20] bases.

It is outside the scope of the present discussion to review the whole field.* It is therefore proposed to deal briefly with the salient features of the fragmentation patterns of indoles, quinolines, quinazolines and *iso*-quinolines with special reference to our recent work on *Ipecac* alkaloids.

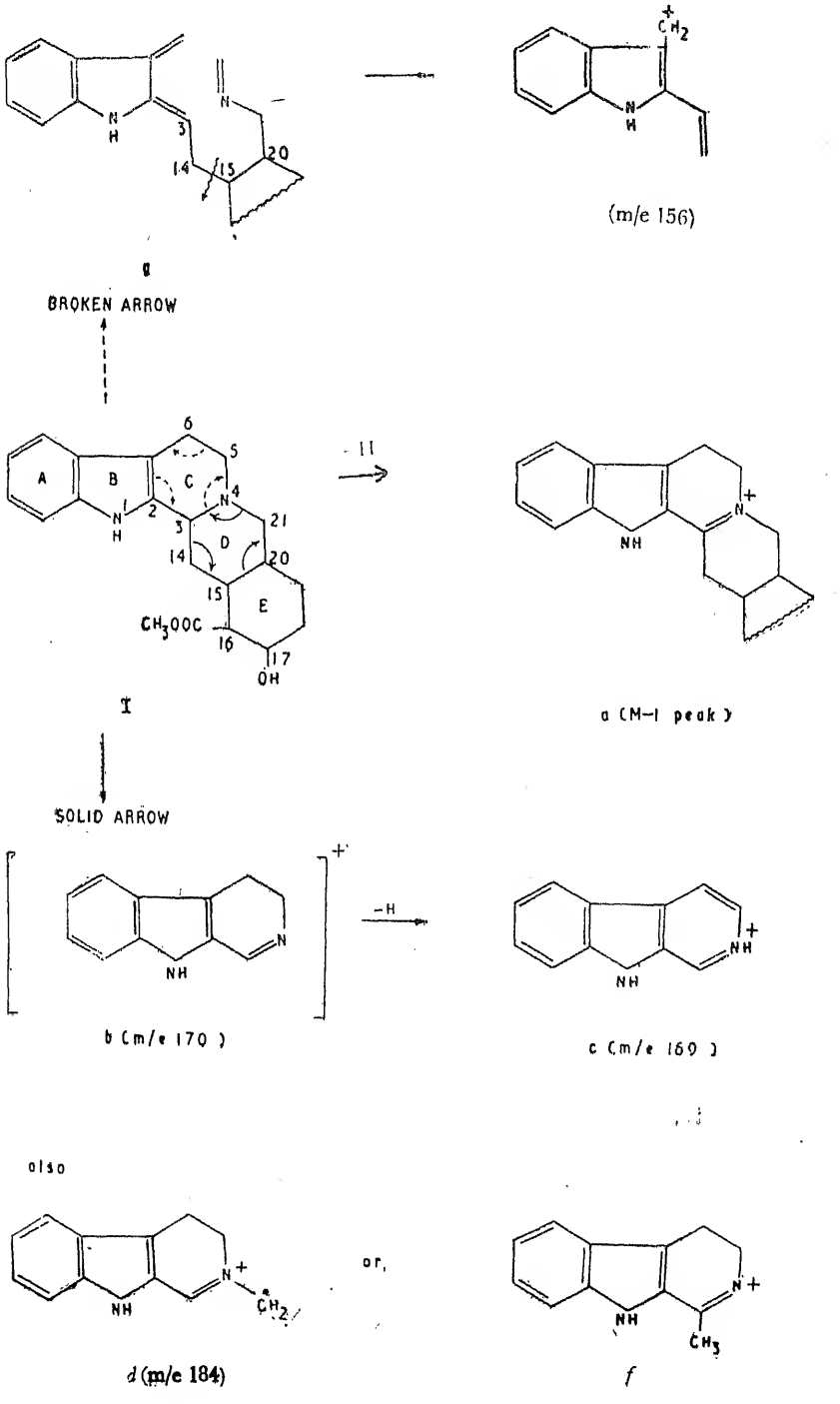
*An excellent treatise has since been published by Prof. Djerassi and his school.[10a].

(a) **Rauwolfia alkaloids :**

(i) *Yohimbane bases :*

The prototype of this group, yohimbine (I), exhibit[21] a M – 1 peak besides those at m/e 184 (*d* or *f*), 170 (*b*), 169 (*c*) and 156 (*e*). The most intense characteristic peak at M-1 is attributed to species '*a*' consequent on the loss of C_3-H, the positive charge being stabilized by conjugation with the indole ring as also by participation of the electron pair on N_b. The genesis of other fragments are shown in Chart 1.

CHART No. 1. - Fragmentation of Yohimbine

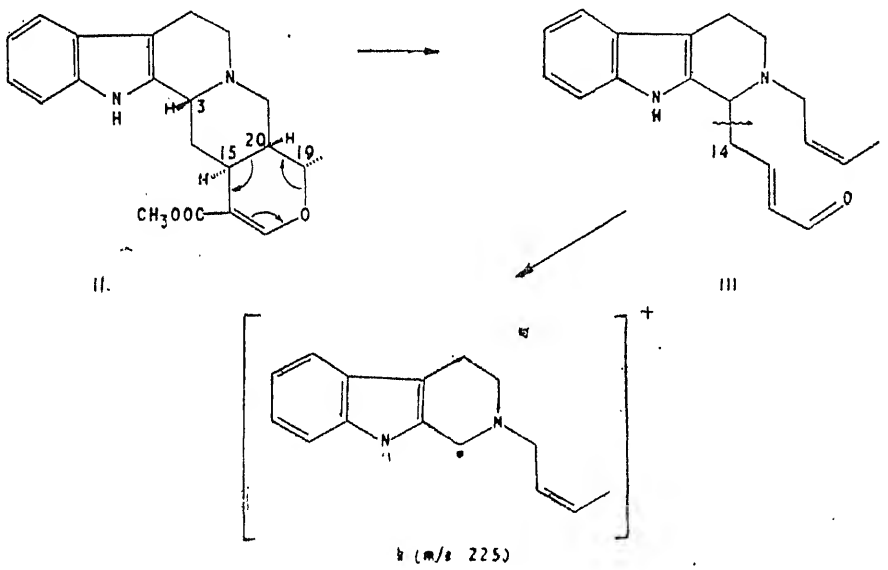


The concerted cleavage (arrows in I) of the allylically activated C_3-C_{14} bond with consequent rupture of N_4-C_{21} bond would result in ion *b* (m/e 170). Further loss of one atom of hydrogen would give well-stabilized ion *c* (m/e 169). Cleavage of C_{20}-C_{21} or C_{14}-C_{15} on the other hand, would lead to the ions *d* or *f* (m/e 184). If, however, the primary fragmentation involves the cleavage of two allylically activated bonds at C_3-N_4 and C_5-C_6 followed by the rupture of allylically labilized C_{14}-C_{15} bond in *g*, species *e* (m/e 156) will result.

The mass spectra of the other yohimbine isomers being almost the same, no stereochemical assignment is possible.

(ii) *Heteroyohimbane bases* :

The typical member, ajmalicine (II) shows identical fragmentation pattern with the remarkable difference in the intensity of the ion peaks at *e* (m/e 156) and *f* (m/e 184). The enhanced intensity of these two peaks may be ascribed to further labilization of the allylically activated bond between C_{15}-C_{15} promoted by the C_{16}-C_{17} double bond. A new peak at m/e 225 *h* may be assumed to have originated as a result of retro Diels-Alder reaction (arrows in II) with subsequent cleavage of the doubly allylically activated 3-14 bond in III.

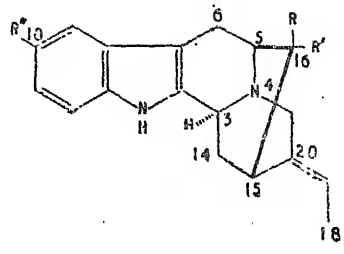


It is interesting to note that the relative intensities of m/e 156, 169, 170 and 184 peaks permit the differentiation of the stereochemistry at the D/E ring junctions of the ring E heterocyclic indoles.

(iii) *Sarpagine type bases* :

Compounds studied[21,22] in this group include besides sarpagine (IV), normacusine-B (V), polyneuridine (VI) and akuammidine (VII). The mass spectrum of sarpagine has been studied[22] in the form of O-methyldeoxydihydrosarpagine (VIII). They also show a strong M – 1 peak though not stronger than that of the molecular ion. Unlike the previous cases this peak probably arises due to abstrac-

tion of hydrogen at C_6 so that the charge could be well-stabilized by conjugation with the indole system and yet the resonance form would not violet Bredt's rule, as would be the case should C_3-H be lost.[21,22] The structures V-VII show very strong peaks at m/e 169 and 168 which are shifted by 30 mass units (m/e 199 (*i*) and 198 (*j*) in VIII corresponding to the increase due to OCH_3 substitution in the aromatic ring.



IV, R = CH_2OH, R′ = H, R″ = OH

V, R = CH_2OH, R′ = R″ = H

VI, R = CH_2OH, R′ = CO_2CH_3, R″ = H

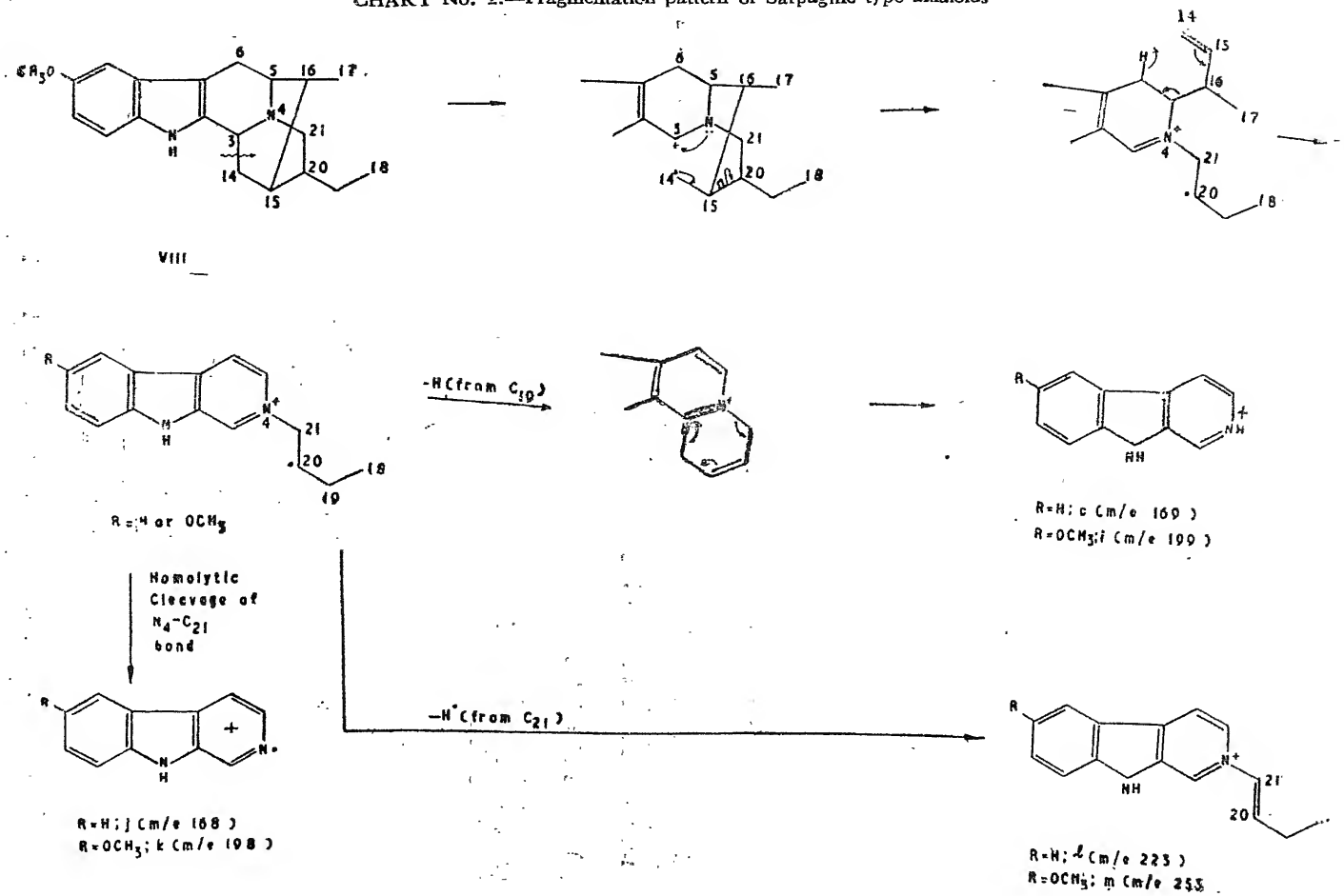
VII, R = CO_2CH_3, R′ = CH_2OH, R″ = H

While m/e 169 must be identical with the carbolinium cation *c* encountered in yohimbane type, m/e 168 (*j*) could be assigned to β-carboline ion itself[22] (Chart 2). In common with the yohimbane type, the initial cleavage occurs at the allylically activated C_3-C_{14} bond.

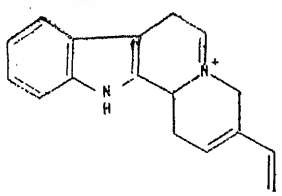
The spectra of the naturally occurring compounds (V-VII) with ethylidene function at C_{20} are distinguished by an intense peak at m/e 249 assigned[21] to species *n*, the 19 : 20-dihydro-derivatives such as dihydronormacusine-B as well as VIII show substantial peak respectively at m/e 223 (*l*) and 253 (*m*) instead (Chart 2). The compounds with ethylidene side-chain also exhibit another less intense peak at m/e 182 (*o*) which corresponds to m/e 184 (*d* or *f*) peak in yohimbane type, and might, arise by way of initial cleavage at the allylically activated bonds at C_{15} and C_{21} centres, followed by loss of one hydrogen atom.

Then again, it has been shown most recently[21a] that the pronounced loss of elements of water (m/e 334) and acetic acid (M – 60) in the spectra of polyneuridine (VI) and its acetate respectively, not observed in akuammidine (VII) or its acetate, provides a simple way of differentiation of the stereochemistry at C_{16} everything else remaining the same. The loss of water or acetic acid has definitely been shown

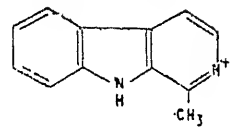
CHART No. 2.—Fragmentation pattern of Sarpagine type alkaloids



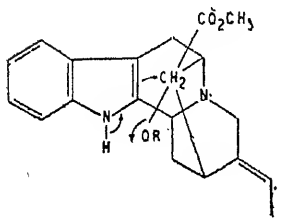
to involve $N_{(a)}$-H and a plausible mechanism has been proposed (VI→IX).



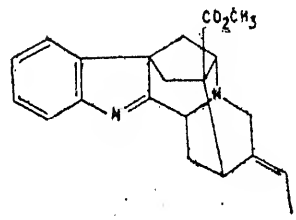
n (m/e 249)



o (m/e 182)



VI, R=H (or Ac)

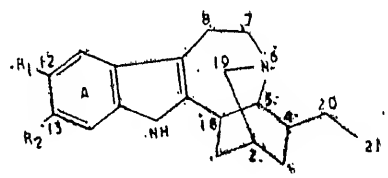


IX

Obviously, such elimination is not expected in compounds containing $N_{(a)}$-CH_3, e. g. voachalotine (N-methyl polyneuridine). Therefore, the fragmentation pattern must depend on whether $N_{(a)}$ is free or substituted.

(b) **Iboga alkaloids :**

Another important group of indole alkaloids, *viz.*, ibogamine (X), ibogaine (XI), tabernanthine (XII) and ibogaline (XIII) have been subjected to mass spectrometric analysis[5].



X, $R_1=R_2=H$

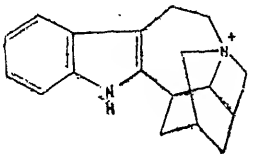
XI, $R_1=OCH_3$; $R_2=H$

XII, $R_1=H$, $R_2=OCH_3$

XIII, $R_1=R_2=OCH_3$

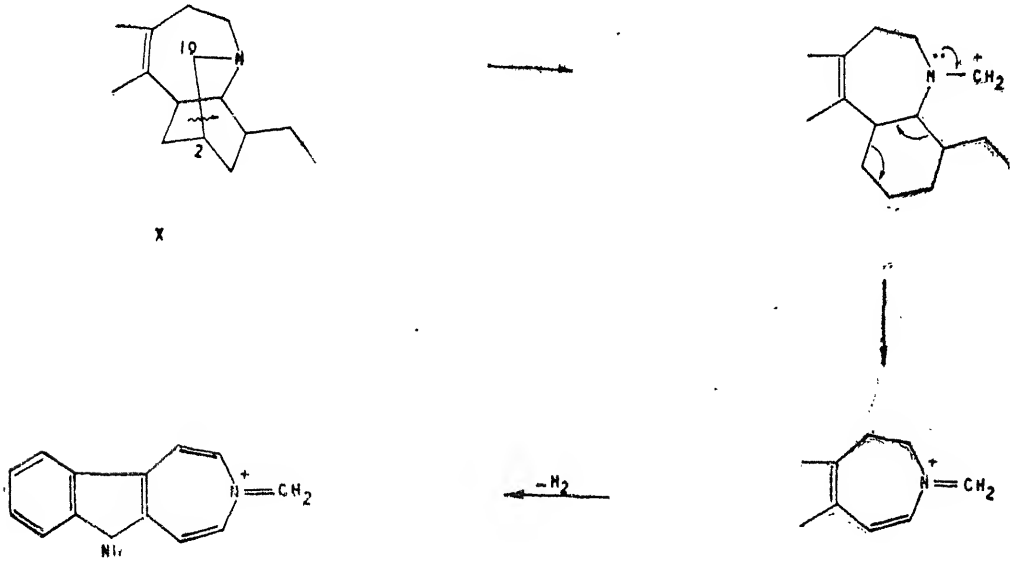
They all show common peaks at m/e 122, 124, 135, 136 and 149 and must have been originated from the common isoquinuclidine moiety whereas the others,

for instance at m/e 156, 195, 251 and 265 besides the M^+ ion at 280 in ibogamine, the simplest member of the group, vary according to the mass of the substituents present in ring A and therefore includes the aromatic part of the molecules. In ibogamine, the peaks at m/e 265 and 251 are clearly due to the elimination of methyl and ethyl groups respectively. It is important to note that from the nature of the fragmentation pattern, it is possible to assign stereochemistry of the side chain in iboga alkaloids. The peak corresponding to the methyl is more intense than that of ethyl which requires *cis* relationship of the ethyl group with respect to N_6. Because, the primary carbonium ion which is otherwise less stable and is formed by the loss of C_{21} is stabilized by the free electron pair at N_6 to form the ammonium ion (XIV) whereas the secondary carbonium ion with a positive charge at C_4 consequent on the loss of ethyl group cannot well be stabilized and hence the more intence peak at m/e 265.



XIV

The peak at m/e 195 (*p*) originates by initial cleavage of C_3-C_{19} bond followed by the concerted rupture of C_{18}-C_1 and C_4-C_5 bonds and concomitant loss of hydrogen atoms from C_7 and C_8 thus

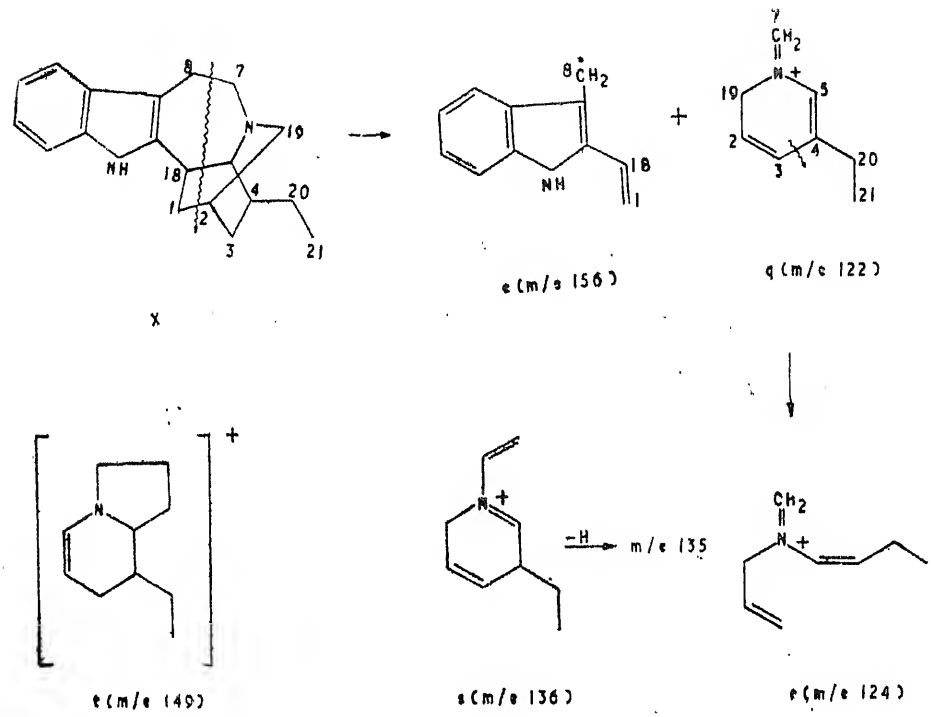


X

p (m/e 195)

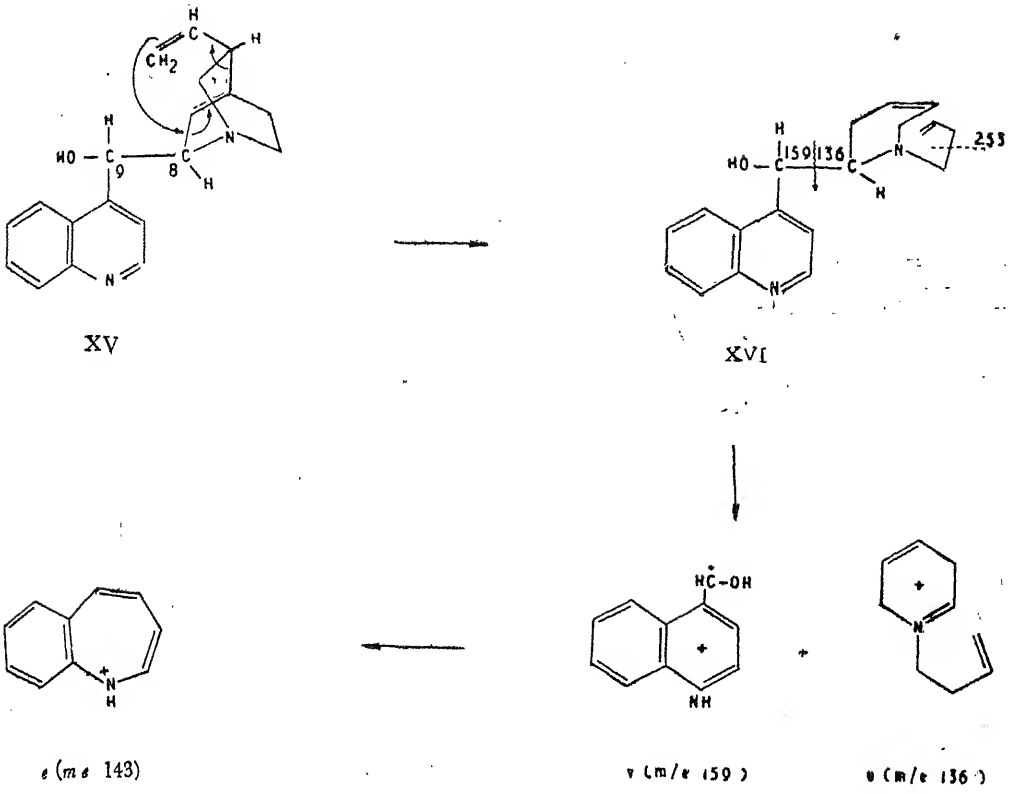
The peaks at m/e 156 may be assigned the same structure as the species (*e*) in yohimbine series. This as well as that at m/e 122 (*q*) might analogously arise by the cleavage of the two allylically activated centres at C_8-C_7 and C_{18}-C_5 followed by allylically labilized C_1-C_2 bond and simultaneous loss of two hydrogens at C_3 and C_4. The simplest representation is shown by the arrow below :

The peaks at m/e 124 (*r*), 136 (*s*) and 149 (*t*) have been tentatively assigned to structures which can be explained by complex mechanism.[5]



The other important group of alkaloids containing quinuclidine moiety is the cinchona alkaloids (quinoline type). A typical member of this group, cinchonine (XV) shows[15] two very strong and characteristic peaks at m/e 136 (*u*, *cf.*, *s*) and 159 (*v*) besides others. The former which has an enormous intensity originates from the quinuclidine and the latter from the quinoline moiety by simple cleavage at the C_9-C_8 bond of XVI, the formation of which from XV is indicated.

The m/e 159 may now lose the hydroxyl group to form the tropylium ion (ω) corresponding to the peak at m/e 143.



ε (m/e 143) τ (m/e 159) υ (m/e 136)

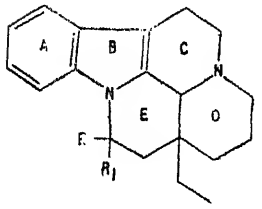
Here again it is possible to differentiate the stereochemistry at C_8 from the intensity of m/e 136 (*u*). For instance, the intensity for quinine and cinchonidine is ten times stronger than those of cinchonine and quinidine.

(c) **Hunteria alkaloids :**

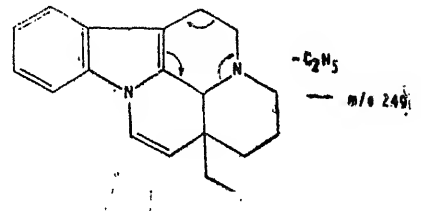
The last type of indole alkaloids that will be discussed is the *Hunteria alkaloids, viz.*, eburnamine (XVII), eburnamonine (XVIII) and eburnamenine (XIX) isolated by Bartlett and Taylor[25] from *Hunteria eburnea* Pichon and later by Schnoes *et al*,[24] isolated them from *Rhazya stricta* as well as from *Aspidosperma quebracho blanco* Schlecht (eubnamenine only). Vincamine (XIX), a *Vinca* alkaloid[25] also fall in this group.

The interesting feature of the mass spectrum[4] of eburnamine (XVII) is that it does not show a molecular ion peak and its spectrum is completely identical with that of eburnamenine (XIX) suggesting dehydration. This could as well be a pointer to the limitation of this method. The simplest member shows very characteristic peaks at m/e 249 and 208 (*x*) apparently due in one case, to the loss

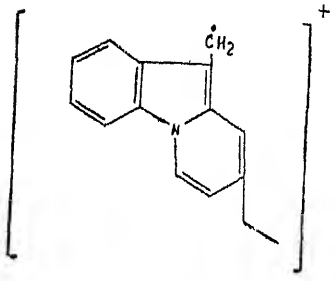
of allylically activated angular ethyl group and in the other by concerted cleavage of ring C (arrows in XIX) followed by a rupture of one allylic bond thus :



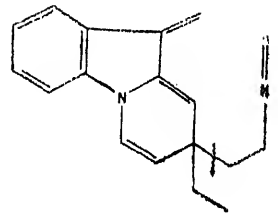
XVII, R=H, R′ =OH
XVIII, R–R′ =O
XX, R= CO_2 CH_2, R′=OH



XIX



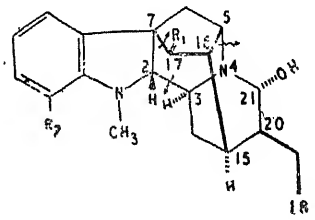
(m/e 208)

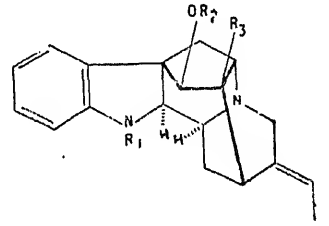


Particular mention must be made to the spectrum of eburnamonine (XVIII). Unlike others in the group, in addition to the peaks corresponding to those mentioned above, it shows a prominent M—1 peak and a strong peak at m/e 237 which could either be ascribed to the loss of carbon monoxide or ethylene (from ring D), the former being preferable.

DIHYDROINDOLE BASES

(a) *Rauwolfia alkaloids* : *Ajmaline type bases.*



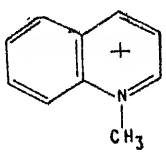


XXI, R_1 = H(OH), R_2 = H
XXII, R_1 = O ; R_2 = H
XXIII, R_1 = O, R_2 = OCH_3

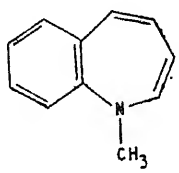
XXIV, R_1 = R_2 = H, R_3 = CO_2CH_3
XXV, R_1 =CH_3, R_2=CH_3CO, R_3=CO_2CH_3
XXVI, R_1=CH_3, R_2=H, R_3=CO_2CH_3
XXVII, R_1=CH_3, R_2=R_3=H; C_2–H=β

The compounds studied in this group are ajmaline (XXI)[26], ajmalidine (XXII), vomalidine (XXIII)[27], quebrachidine (XXIV), vincamedine (XXV), vincamajine (XXVI) and tetraphyllicine (XXVII)[28].

Ajmaline, the first to be studied in this group, shows[26] characteristic peaks at m/e 144, 157, 182 and 183 besides peaks at M−15 (loss of CH_3), M−29 (loss of C_2H_5) and the molecular ion at m/e 326. While the peaks at m/e 183 and 182 were assigned to the β-carbolinium cations corresponding to species *c* (m/e 169) and *j* (m/e 168) from yohimbine, the peaks at m/e 144 and 157 were attributed to the quinolinium and tropylinium ions *y* and *z*, respectively. The formation of the last two could involve rupture of the bonds C_7-C_{17}, C_2-C_3 and N_4-C_5 (arrows in XXI) followed by rearrangement and ring expansion. It thus appears that the carbolinium cation *c* with due regard to the substituents in the indole moiety is the common fragment of all the β-carboline derivatives.



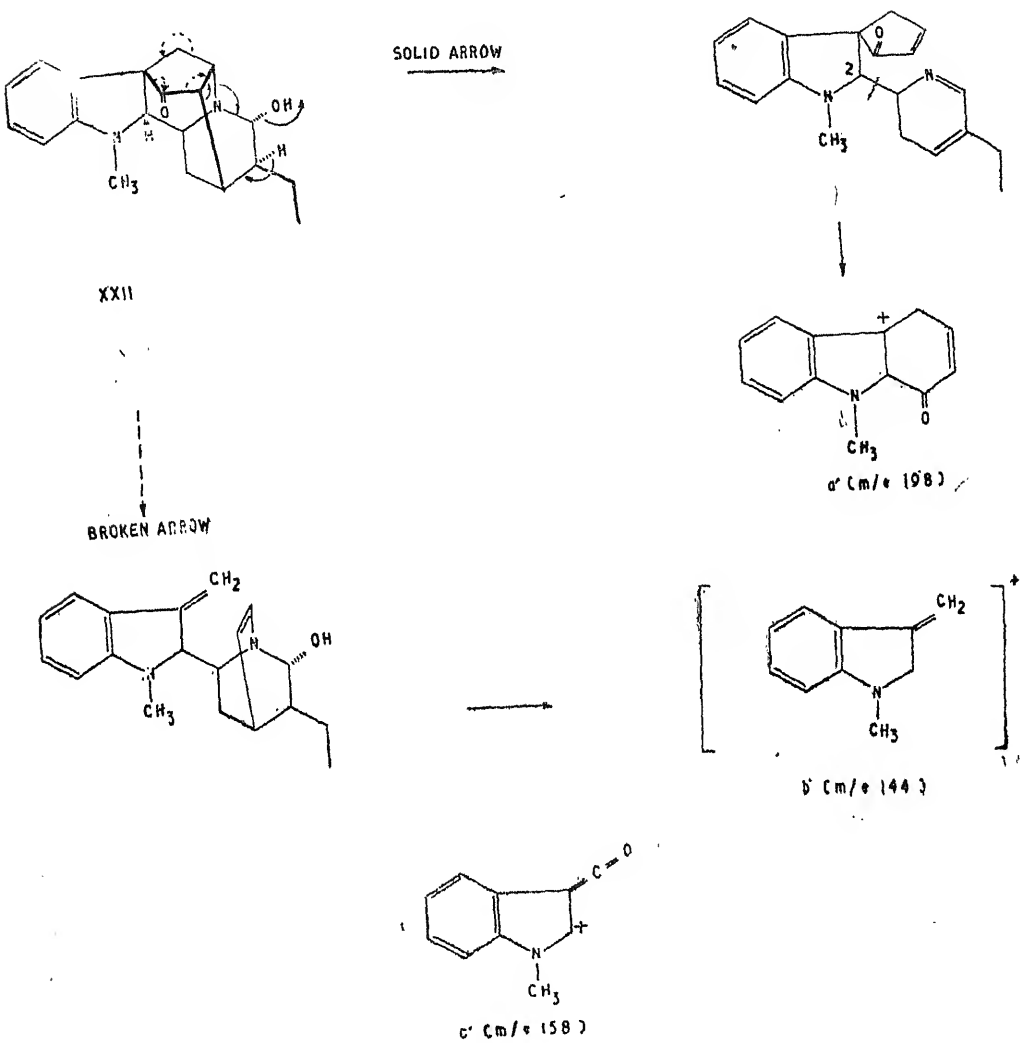
y (m/e 144)



z (m/e 157)

However, Biemann *et al.*[27], as a result of their recent studies on the same system with high resolution mass-spectrometry came to the conclusion that difference in substitution and/or stereochemistry at the alicyclic ring system sometimes radically change the fragmentation pattern. For instance in ajmalidine (XXII), the peaks at m/e 183 and 182 characteristic of ajmaline are of very low intensity while intense peaks appear at m/e 198 (*a'*) 144 (*b'*) and a doublet at m/e 158 (*c'*) corresponding to the compositions $C_{13}H_{12}NO$, $C_{10}H_{10}N$ and $C_{10}H_8NO$ as well as $C_{11}H_{12}N$, respectively. The fragment *a'* could arise by elimination of water, retro

Diels-Alder cleavage followed by rupture of C_2-C_3 bond with subsequent expansion of the spiro-ring for stabilizing the positive charge thus :



Alternate elimination of CO of the cyclopentanone ring as shown by broken arrow could lead to fragment $C_{10}H_{10}N$ corresponding to m/e 144 (*b'*), the most intense peak in the whole spectrum. Obviously, this process is simply impossible with ajmaline molecule.

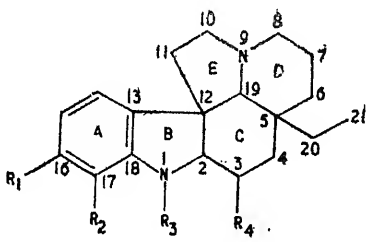
The fragment corresponding to $C_{10}H_8NO$ at m/e 158 (*c'*) must contain the C_{17}-oxygen function along with the indole moiety. This fragment, exhibited by all the compounds of this type studied so far, is considered to be quite diagnostic of ajmaline ring system. The spectrum of vomalidine (XXIII) supports all these assignments.

It has been concluded by Biemann *et al.* (*loc. cit*) that all the ajmaline derivatives with or without the presence of one or both hydroxyl function at C_{21} and at C_{17} irrespective of the stereochemistry at the latter centre behave similarly. However, a keto function at C_{17} in ajmaline modifies (*vide supra*) and epimerization at C_2 radically changes the fragmentation pattern. Thus, quebrachidine (XXIV), an *Aspidosperma* alkaloid of the epi-series having a mass of 352 shows[28] key fragments at m/e 222, 190, 143 and 130. The last two have been assigned to indole moiety with two and one carbon atoms respectively (*vide infra*). Loss of 130 mass units clearly leads to the fragment at m/e 222 which by further loss of 32 mass units in the form of CH_3OH may give rise to the peak at m/e 190.

Therefore, mass spectrum can easily distinguish the stereochemistry at C_2 in ajmaline type of compounds.

(b) *Aspidospermidine type alkaloids* :

Mass spectrometry has found most extensive application in this field. The compounds so far studied include XXVIII—XXXVII.



XXVIII, $R_1=R_2=R_3=R_4=H$; Aspidospermidine

XXIX, $R_1=R_2=R_3=H$, $R_4=CO_2CH_3$; Dihydrovincadifformine

XXX, $R_1=R_2=R_4=H$, $R_3=CH_3$; 1-Methylaspidospermidine

XXXI, $R_1=R_3=R_4=H$, $R_2=OCH_3$; Deacetylaspidospermine

XXXII, $R_1=R_4=H$, $R_2=OCH_3$, $R_3=CH_3$; 1-Methyldeacetylaspidospermine

XXXIII, $R_1=R_4=H$, $R_2=OCH_3$, $R_3=CH_3CO$; Aspidospermine

XXXIV, $R_1=R_2=OCH_3$, $R_3=R_4=H$; Deacetylpyridifoline

XXXV, $R_1=R_2=OCH_3$, $R_3=CH_3CO$, $R_4=H$; Pyrifolidine

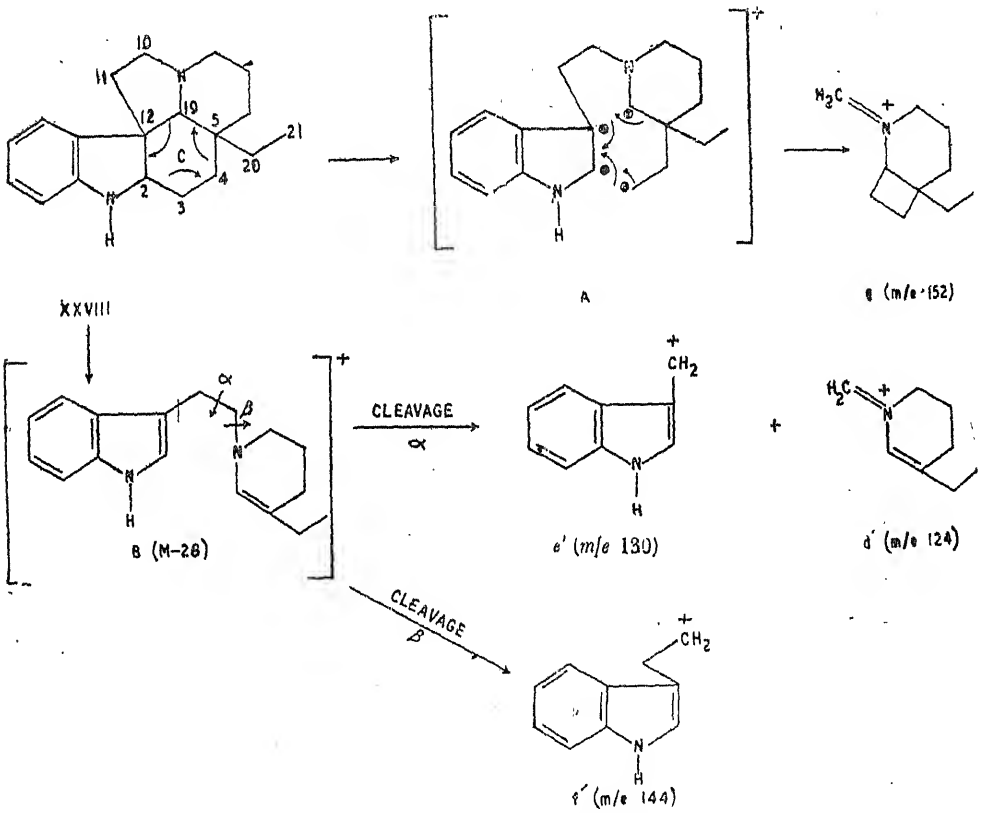
XXXVI, $R_1=H$, $R_2=R_4=OH$, $R_3=CH_3CO$; Spegazzinine

XXXVII, $R_1=R_2=R_4=OH$, $R_3=CH_3CO$, Spegazzinidine

Aspidospermidine (XXVIII)[12], the simplest member, shows important peaks at m/e 124, 130, 143, 144, 152, 254, M−28 and 282 (M^+). Biemann *et al.*[30] were the first to observe that the one at M−28 and particularly the most intense peaks at m/e 124 are diagnostic of an aspidospermine skeleton, the relative abundance of the former being dependent on the nature of substituents in the indole moiety.

The genesis of the peak at M—28 requires explusion of C_3 and C_4 as ethylene by concerted rupture of ring C initiated at the benzylically activated bond, C_{12}-C_{13} connected to N_9, and thus relieving strain inherent in such a condensed system. However, the appearance of a peak at m/e 152 (*g'*) requiring retention of C_3-C_4 on the piperidine ring led Biemann *et al*[12] to suggest that the cleavage of ring C is not completely concerted. According to them, initial cleavage of C_{12}-C_{19} and C_2-C_3 bonds may give rise to a diradical (*A*) which though more usually eliminate C_4-C_5 bond resulting in the species *B* (M—28), occasionally form a C_3-C_{19} bond. Subsequent cleavage at C_{10}-C_{11} of these two species can now lead to the ions at m/e 124 (*d'*) and 152 (*g'*) respectively.

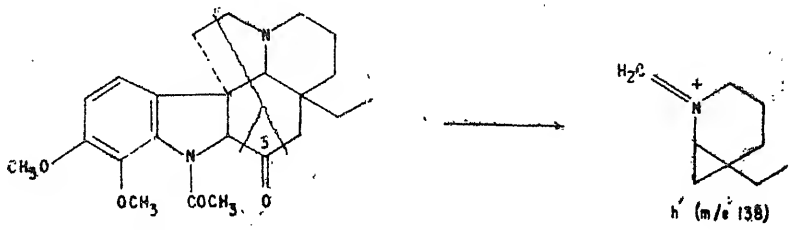
The absence of M – 29 peak shows that the elimination of ethyl side chain as such is unlikely because it would lead to a very unfavourable bridgehead carbonium ion



The cleavage of species *B* at C_{10}-C_{11} and C_{10}-N_9 bonds leads to the indole fragments *e'* (m/e 130) and *f'* (m/e 144) both being common to all the naturally occurring dihydroindole alkaloids without any substituent in the indole moiety. The peak at m/e 143 may arise by a loss of hydrogen atom from species *f'*.

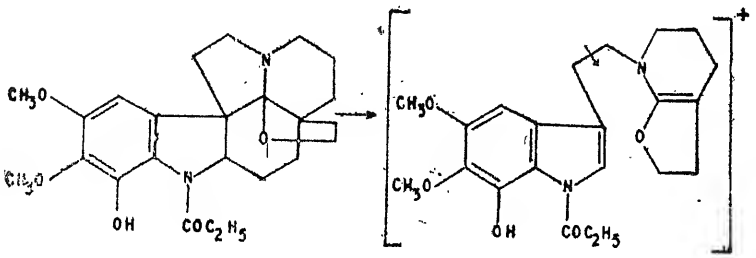
Since in a normal aspidospermine-like fragmentation, the C_3 and C_4 centre are expelled, the nature of the substituents present at these two sites could be anticipated from the shift of M—28 peak. For example, spegazzinine (XXXV. exhibits a peak at M – 44 and dihydrovincadifformine (XXIX) at M – 86 due to the expulsion of C_3 and C_4 bridge as ethyl alcohol and methyl acrylate respectively[4],

For the same reason, the genesis of m/e 124 (*d'*) is independent of the substituents at C_3 and C_4 unless C_2-C_3 bond is unsaturated (*e.g.*, vincadifformine, LVII) or a keto function is present at C_3. For instance, a ketone (XXXVIII) derived from spegazzinidine (XXXVII) shows[32] an intense peak at m/e 138 (*h'*) in lieu of that at m/e 124 and its genesis may be visualized as follows :

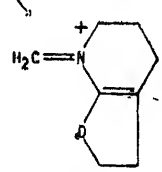


XXXVIII

h' (m/e 138)



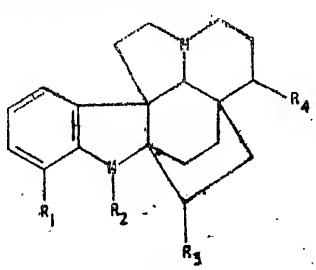
XXXIX



i' (m/e 138)

The same m/e 138 peak observed in aspidoalbine (XXXIX)[33], wherein a tetrahydrofuran ring is attached to ring D, however, should be assigned to the species *i'*. Substitution in ring D could also be recognized in pyrifoline (XLI)[6], a representative of a fairly large group of interesting compounds with hexacyclic skeletal structure in which the angular ethyl group of aspidospermine is a part of a carbocyclic ring.

(c) *Aspidospermine type with additional ring* :



XL, $R_1=OH, R_2=COCH_3, R_3=R_4=H$

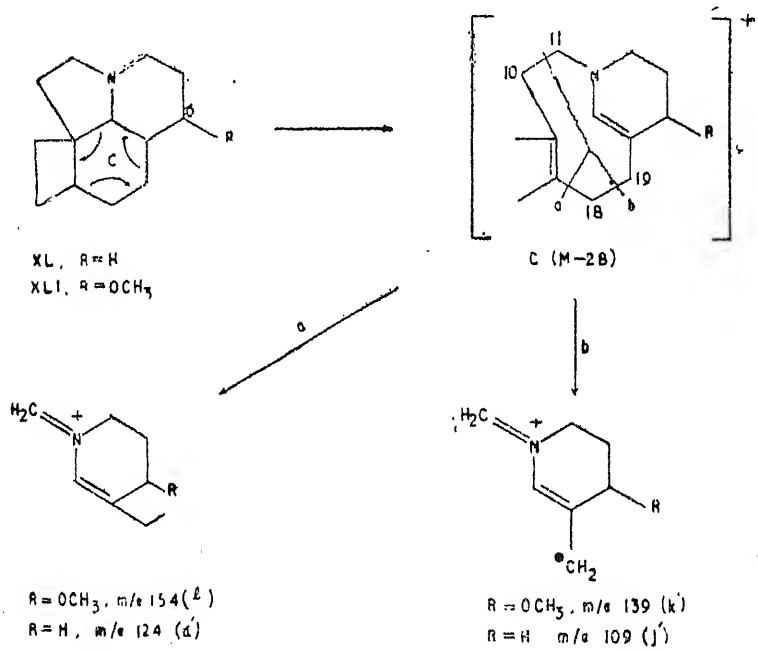
XLI, $R_1=R_4=OCH_3, R_2=COCH_3, R_3=H$

XLII, $R_1=OCH_3, R_2=CHO, R_3=CO_2CH_3$

XLIII, $R_1=R_3=H, R_2=CHO, R_4=OCH_3$

XLIV, $R_1=R_4=H, R_2=CHO, R_3=CO_2CH_3$

The compounds studied in this group besides pyrifoline are aspidofiline (XL)[34], refractine (XLII)[11], refractidine (XLIII)[6], aspidofractine (XLIV)[35]. They all show M−28 peak indicating expulsion of C_3-C_4 as unsubstituted ethylene group. Aspidofiline, having no substituent in ring D, shows a very strong peak at m/e 109 (*j'*) in addition to m/e 124 (*d'*) peak. The greater intensity of the former which appears to be characteristic of this system is definitely due to the more favourable cleavage at the two allylic centres at C_{10}-C_{11} and C_{18}-C_{19} in species *C*. Corresponding peaks of pyrifoline appear at m/e 139 (*k'*) and 154 (*l'*)—a difference of 30 mass units—due to additional OCH_3 function in ring D as shown below.

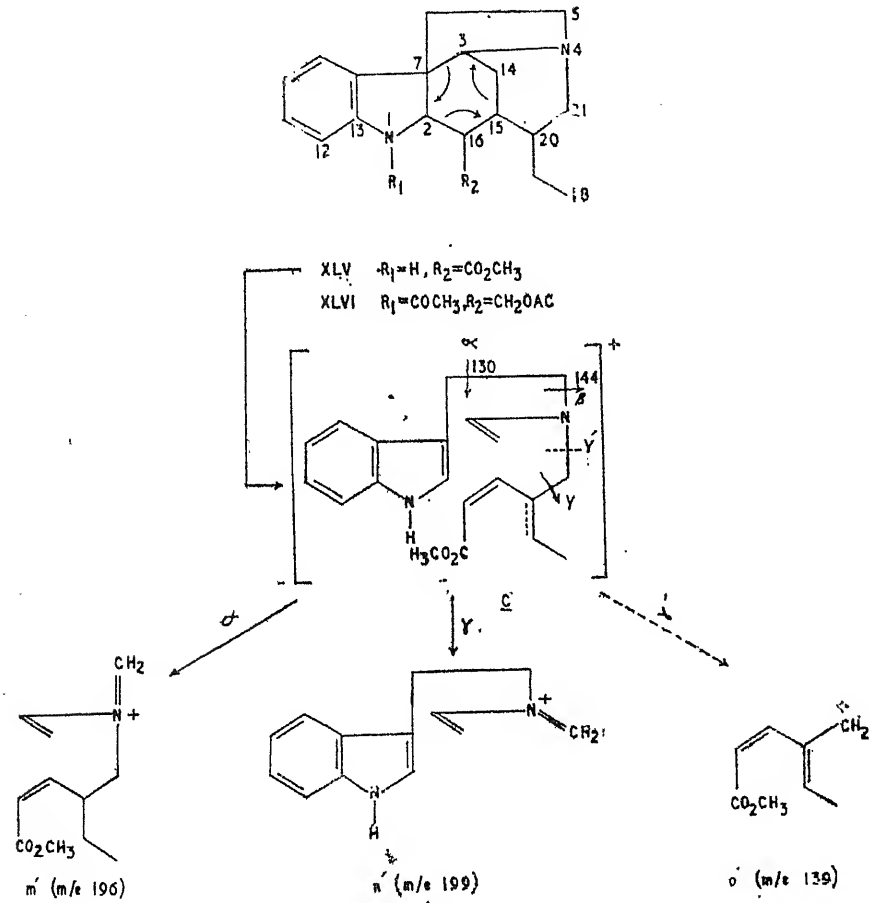


It may be pointed out that when present, the acetyl function at N_1 is expelled[14,30] as a ketene $(M-42)^{+\cdot}$.

(d) *Tetrahydroakuammicine type*:

Another important type of dihydroindole alkaloids having tetrahydroakuammicine (XLV) structure is represented by the naturally occurring geissochizoline (XLVI)[36]. Here also the fragmentation starts at ring C. Tetrahydroakuammicine itself shows important peaks at m/e130, 144, 196 and 199. The first two represent indole nucleus with one and two carbons (species *e'* and *f'*). Assigments to other peaks as shown below have been justified by Budzikiewicz *et al.*[14] on the basis of "mass spectrometric shift" technique which also help determine the nature of substitution at C_{12}, N_1, C_{16}, C_{14} and C_{20}.

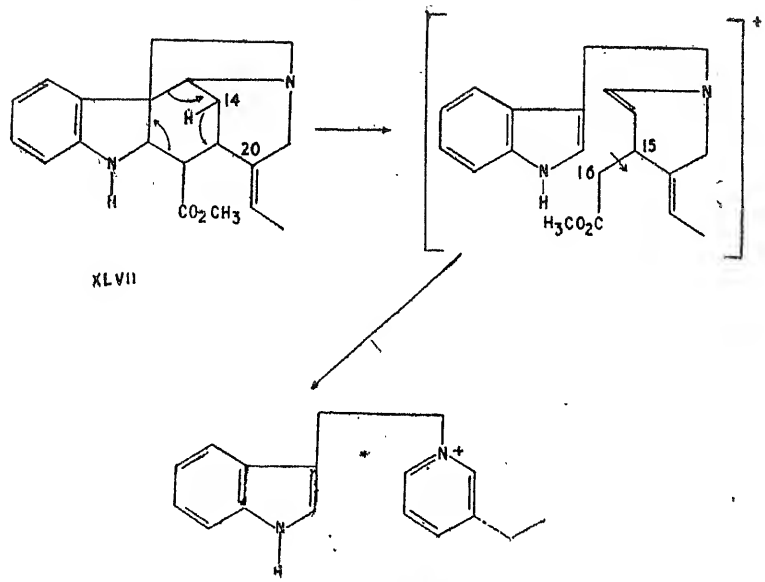
The most important observation they made was that the fragmentation pattern is greatly influenced by the presence of a ethylidene function at C_{20} (2 : 16-dihydroakuammicine type) or at C_{14} (aspidospermatine type) and the presence of the double bond at C_2-C_{16} (α-methyleneindolenine type).



(d) *2 : 16—Dihydroakuammicine type* :

The peak at m/e 199 (*n'*) disappears and two additional peaks at m/e 139 (*o'*) and 251 (*p'*) appear instead in 2 : 16-dihydroakuammicine (XLVII). The change is obviously due to the introduction of a double bond at C_{19}-C_{20} which now activates C_{21}-N_4 centre where the cleavage occurs rather than at C_{20}-C_{21} bond to produce the fragment *o'* (*via* path γ' in C). The formation of species *p'* involves expulsion of C_{16}-carbon atom along with its substituent and additional hydrogen atom. Budzikiewicz *et al.* (*loc. cit*) rationalized the genesis of this fragment by

assuming rupture of ring C with hydrogen transfer from C_{14} to C_{16} as shown below :

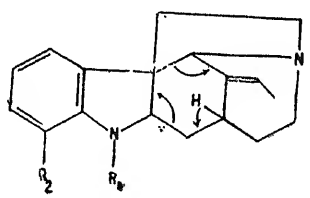


XLVII

p' (m/e 251)

(e) *Aspidospermatine type* :

The compounds studied in this group include XLVIII—LII.



XLVIII, $R_1=R_2=H$, Aspidospermatidine

XLIX, $R_1=CH_3$, $R_2=H$, 1-Methylaspidospermatidine

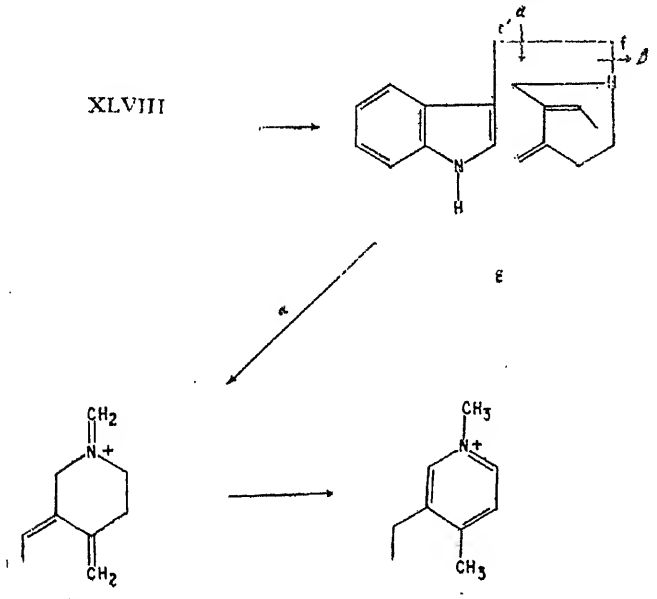
L, $R_1=H$, $R_2=OCH_3$, Deacetylaspidospermatine

LI, $R_1=CH_3CO$, $R_2=H$, 1-Acetylaspidospermatidine

LII, $R_1=CH_3CO$, $R_2=OCH_3$, Aspidospermatine
(and 14 : 19-Dihydroaspidospermatine).

Aspidospermatidine (XLVIII), the simplest member of the series exhibit[12] the most characteristic peak at m/e 136 (*q'*) in addition to those for indole fragments at m/e 130 (*e'*) and 144 (*f'*).

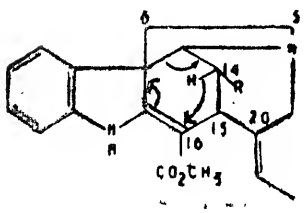
Budzikiewicz et al.[14] have fully discussed the difficulty in assuming usual tetrahydroakuammicine type fragmentation of ring C envisaged by Biemann *et al.*[12] The former group of workers presume hydrogen transfer from C_{15} to C_3 as the initial step followed by the usual fragmentation to give the ion at *q'* which can then rearrange to yield the highly stabilized aromatic ion *r'* as shown below :



q (m/e 136)

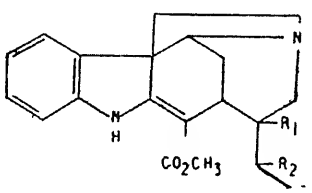
(f) *α-Methyleneindolenines* :

(i) *Akuammicine type* : Compounds studied in this group are LIII—LVI.



LIII, R=H, Akuammicine

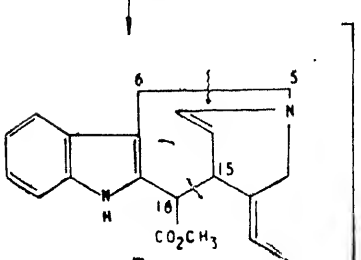
LIV, R=OH, Mossambine



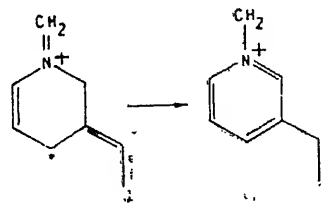
LV, R_1=OH, R_2=H; Lochneridine

LVI, R_1=H, R_2=OH; Echitamidine

when R=H

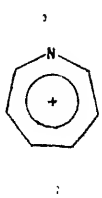


F



t' (m/e 121)

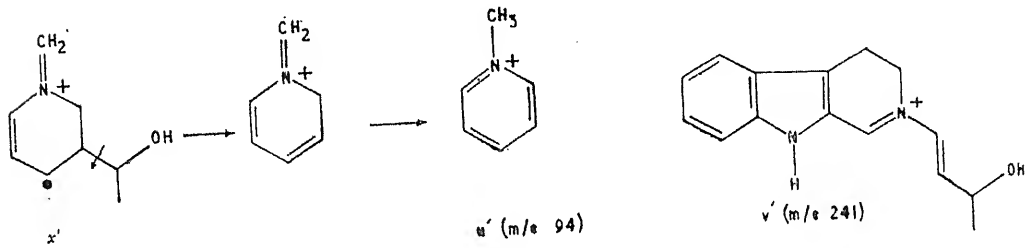
u'



v' (m/e 92)

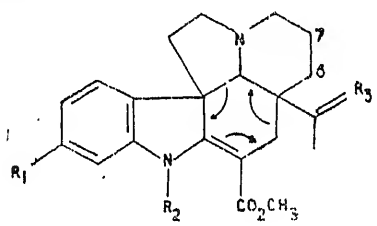
Akuammicine (LIII) shows intense peaks at m/e 92 (s') 107 and 121 (t') while echitamidine (LVI) exhibit an additional peak at m/e 94 (u') and 241 (v'). Simple retro-Diels-Alder transformation being not possible in akuammicine, the ring C can open up all the same by transfer of C_{14}-H to C_{16}. The resulting ion F can now be cleaved at the allylically labilized bonds at C_5-C_6 and C_{15}-C_{16} yielding species t' which in turn may rearrange to the stable aromatic structure w'. The m/e 92 and 107 peaks have been assigned to the azatropylium ions (s') and N-methylazacycloheptatriene cation.

The fragment (x') from echitamidine corresponding to specise t' has now a saturated side chain which may be lost to furnish the stable pyridinium ion u' (m/e 94) thus :



The mode of formation of the peak at m/e 241 from echitamidine is difficult to visualize although however it has been tentatively assigned to the species v' based on speculative machanism[14] we need not go into the details.

(ii) *Aspidospermidine type* :



LVII, $R_1=R_2=H$, $R_3=H_2$

LVIII, $R_1=R_2=H$, $R_3=H_2$; $\triangle$ 6 : 7

LIX, $R_1=H$, $R_2=CH_3$, $R_3=H_2$

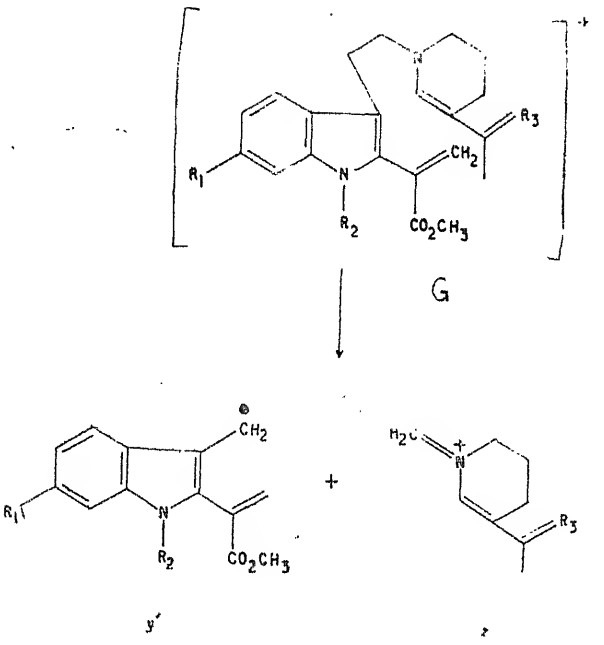
LX, $R_1=R_2=H$, $R_3=O$

LXI, $R_1=OCH_3$, $R_2=H$, $R_3=O$

LXII, $R_1=R_2=H$, $R_3=H$ (OH)

Compounds studied in this group comprise vincadifformine (LVII)[31] tabersonine (LVIII)[37], minovine (LIX), minovincine (LX), 16-methoxyminovincine (LXI) and minovincinine (LXII)[38]

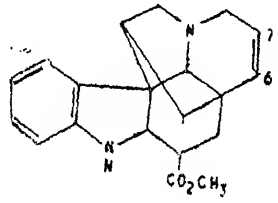
The retro-Diels-Alder fragmentation of ring C leads to ion *G* which further fragments to species, *y'* and *z'*.



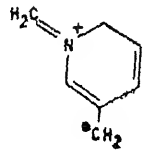
For instance in vincadifformine (LVII)[31] *y'* correspond to m/e 214 and *z'* is identical with species *d'* (m/e 124). The latter fragment which is very characteristic of aspidospermidine skeletal structure is also very intense in vincadifformine.

Though the 6 : 7-dihydrotabersonine shows typical fragmentation pattern of vincadifformine, tabersonine (LVIII) itself exhibits peaks at m/e 92, 107 and 135 arising out of the unsaturated piperidine moiety. In addition to those, 2 : 3-dihydrotabersonine exhibits peaks at M—86 (m/e 252) due to loss of methylacrylate and at m/e 121 and 122 characteristic of vindolinine (LXIII)[39] having a 6 : 7-

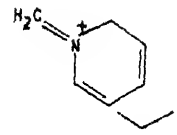
double bond in an analoguous system. The peaks at m/e 107, 135 and 122* have been assigned to species a'', b'' and c''.



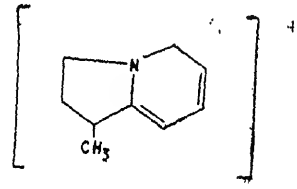
LXIII



a'' (m/e 107)

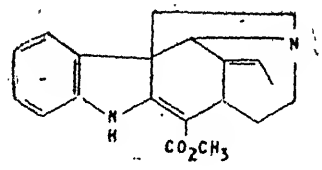


c'' (m/e 122)



b'' (m/e 135)

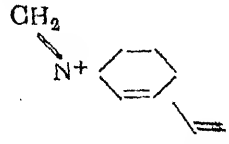
(iii) *Aspidospermatine type* :



LXIV

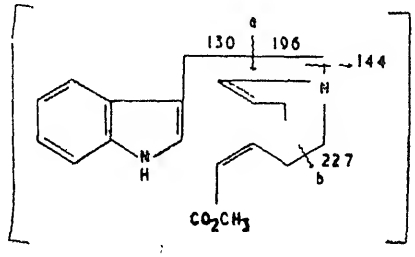
Unlike akuammicine type, the peaks at m/e 92 (s') and 107 are absent in condylocarpine (LXIV)[49]. Apparently the presence of ethylidene function at C_{14} affects the fragmentation pattern of ring C. However, the tetrahydro-derivative

*Budzikiewicz *et al.*[10a] later assigned the following structure to this species.



(*m/e* 122)

shows peaks at m/e 130 and 144, characteristic of dihydroindole alkaloids, a short peak at m/e 227 that serves to differentiate dihydro-aspidospermatine from tetrahydro-decarbomethoxyakuammicine and an intense peak at m/e 196. The fragmentation pattern of tetrahydrocondylocarpine is summarized in the expression *H*.

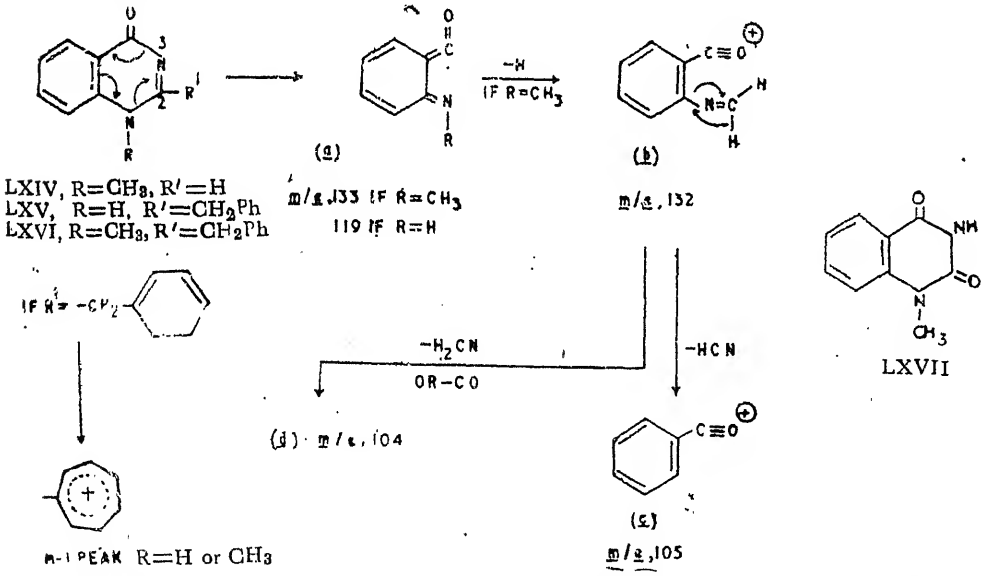


H

The mass spectra of α-methyleneindolenines together with its 2 : 16-dihydro- and 2 : 16 : 19 : 20-tetrahydro-analogues have helped solve many a structural problems[14].

QUINAZOLINE ALKALOIDS

We shall now turn towards the consideration of our own work[8] on the four quinazoline alkaloids isolated from *Glycosmis arborea* (Roxb.)DC, *viz.* glycorine (LXIV), glycosminine (LXV), arborine (LXVI) and glycosmicine (LXVII). Their fragmentation pattern is shown below* :



The spectra shows interesting features. All of them show common peaks at m/e 92, 90, 78 and 77 (*e-h*) and must involve the common aromatic part. Of course,

*See however, Budzikiewicz *et al*[10a]. Monograph, Chapter 15 for the modification of initial fragmentation pattern.

the most important common peak appear at *a* (at m/e 119 when N_1 is unsubstituted with an expected shift to m/e 133 when it is methylated) and can thus be treated as the characteristic fragment of quinazoline class of compounds. Again the N_1-methylated compounds show common peaks at *b* (m/e 132), *c* (m/e 105) and *d* (m/e 104) which are conspicuosly absent in the one with a free NH. The most remarkable feature of those carrying a benzyl substituent (*e.g.*, glycosminine and arborine) are that they show pronounced M—1 peak (intensity much greater than that due to M^+ ion) and a short peak at m/e 91.

In general, the initial fragmentation of the quinazoline alkaloids involves the loss of the atoms at 2 and 3 along with their substituents by concerted cleavage (arrows in LXIV) leading to the ion '*a*' which might lose a hydrogen atom to furnish species *b*.

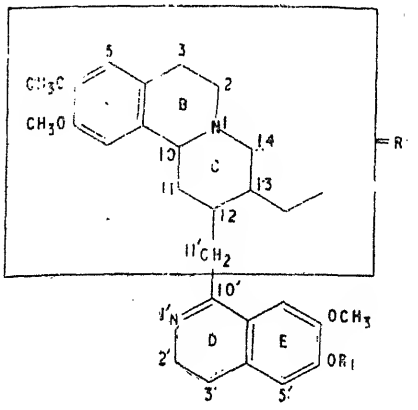
The peaks at m/e 105 and 104 may well involve loss of either CO or $N{=}CH_2$ both having the same mass (28 units) accompanied by rearrangement of hydrogen where necessary. However, expulsion of HCN from the species *b* whereby both the neutral fragment and the resulting ion would be well-stabilized seems to explain the formation of m/e 105 (*c*) better. On the other hand, elimination of a molecule of CO would be more desirable in accounting for the peak at m/e 104 (*d*). Now, the simultaneous appearance of M—1 and m/e 91 peaks in glycosminine (LXV) and arborine (LXVI) must be associated with the benzyl group which can easily split off a hydrogen and rearrange to highly stabilized tropylium ion. We have already encountered the tropylium ion in the other class of alkaloids already discussed.

ISOQUINOLINE ALKALOIDS

We were specially interested in this field because it has been possible for us to isolate[18] emetine, cephaeline and psychotrine, the *Ipecac* alkaloids from the seeds of *Alangium lamarckii* Thw. (Alangiaceae). Incidentally, this represents the first recognition of the structure of any alkaloid reported from this source and the only source for this type of alkaloids discovered so far outside *Ipecacuanha*.* It should be mentioned that though simultaneously done, the mass spectrum of emetine has already been published by a separate group of workers[15] independently with the assignment of the peak at m/e 246 to a wrong species.

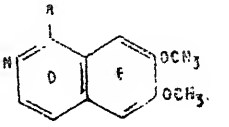
*This information was earlier disclosed by the author in the Symposium on "*Physical methods in Organic Structure Determination*" held at the National Chemical Laboratory, Poona during December 5-7, 1963.

The well-known *Ipecac* alkaloids are derived from three basic types (LXVIII, LXIX and LXXI) differing only in the degree of saturation in ring D.

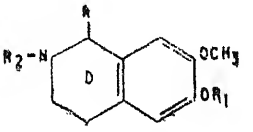


LXVIIIa, R_1=H ; Psychotrine

b, R_1=CH_3, Psychotrine methylether



LXIX Emetamine



LXXa, R_1=R_2=H ; Caphaeline

b, R_1=H, R_2=CH_3 ; N-methylcephaeline

LXXIa, R_1=CH_3, R_2=H ; Emetine

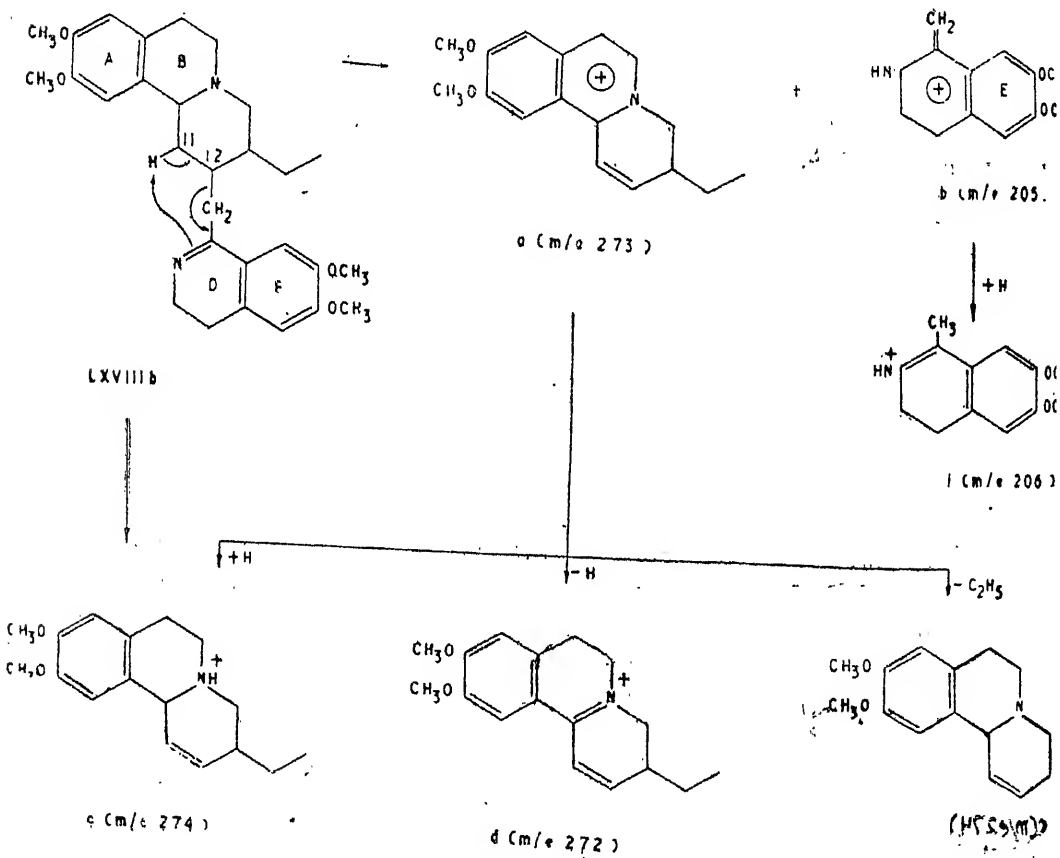
b, R_1=R_2=CH_3 ; N-methylemetine

All of them show distinct molecular ion, peaks at M−15 and M−29 consequent on the loss of the methyl and ethyl groups respectively from the side chain. Common peaks observed at m/e 274 (*c*), 272 (*d*) and 190-192 (*f,g,h*) must have originated from the common ABC ring system. The additional fragmentation is of course dependent on the nature of ring D thus allowing differentiation between the types on the basis of mass spectrum. It may be noticed that the presence of the two isoquinoline rings, sometimes identically substituted, render the interpretations of the fragmentation processes difficult. To obviate this difficulty, the free phenols as well as their corresponding methyl ethers have been studied. Now emetamine (LXIX) differs from psychotrine methylether (LXVIIIb) in having an additional double bond in ring D. Apart from the expected mass difference (*viz.* m/e 203, 204

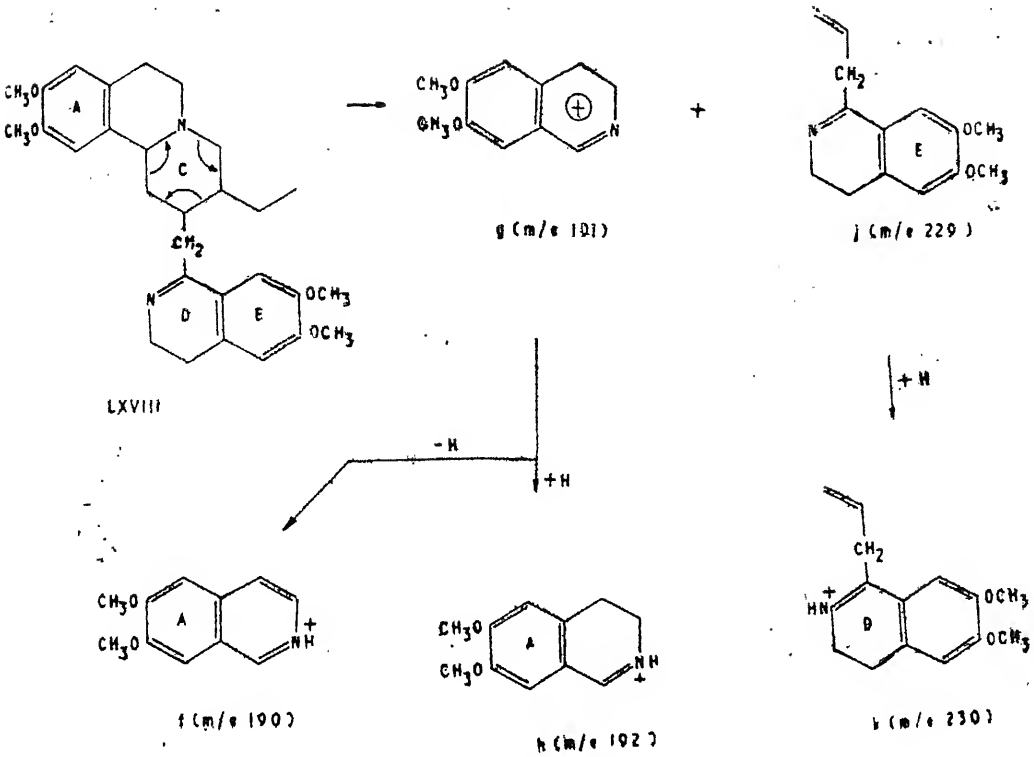
and 228 in the former is shifted 2 mass units higher in the latter) at the appropriate fragments involing rings D and E, both of them virtually show the same spectrum though the intensity differences of the peaks are marked.

Psychotrine methylether (LXVIIIb) shows a number of characteristic peaks. The most intense ones appear at *a* to *e*. The peaks at *a* (m/e 273), *b* (m/e 205) and *c* (m/e 274) may originate through the cleavage of the methylene bridge at C_{11}-C_{12} (accompanied by rearrangement of C_{11}-H to nitrogen for fragment *c*). The fragment *d* (m/e 272) as well as *c*, both providing satellite peaks for *a*, most possibly represent the stabilized species of fragment *a*. The latter may easily expel the ethyl side chain promoted by the allylic double bond to furnish the fragment *e* (m/e 244).

Concerted cleavage of ring C (arrows in ring C in LXVIIIb shown below) can ead to ion *g* (m/e 191) that could likewise stabilize either by separation or addition of a hydrogen atom and the corresponding satellite peaks could also be observed at *f* (m/e 190) and *h* (m/e 192) while the fragment *i* (m/e 206) and *k* (m/e 230)



conceivably represent the protonized fragment of *b* (m/e 205) and *j* (m/e 229) respectively.

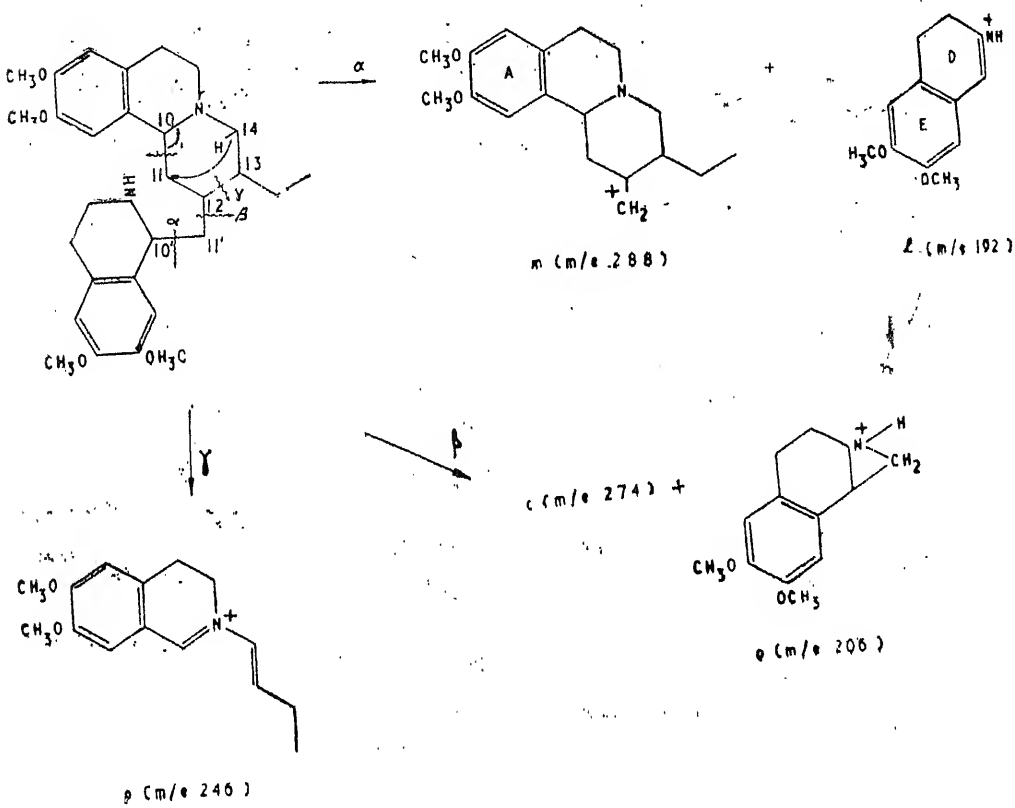


The type III represented by emetine (LXXIa) shows important peaks at m/e 288 (*m*), 246 (*p*), 206 (*o*) and 192 (*l*) but the peaks at m/e 273 (*n*) and 244 (*s*) are conspicuously absent.

The cleavage at C_{10}'-C_{11}' is particularly favoured because this bond is benzy-

lically activated giving rise to fragments at m/e 288 and 192. The cleavage at this bond is prevented in the other type because of the N_1'-C_{10}' double bond in ring D. The ion at m/e 192 being extremely stabilized shows rather the most intense peak in the whole spectrum whereas m/e 288 being a primary carbonium ion is far less stabilized which explains its low intensity.

Splitting at C_{11}'—C_{12} bond would lead to m/e 206 in one part and m/e 274 (*e*) on the other. The formation of ion m/e 246 involves splitting of bonds C_{12}-C_{13} and C_{10}-C_{11} thus :



In conclusion it may be remarked that the application of mass spectrometry is still in its developing stage. However, the foregoing discussions adequately illustrate the power of this new tool.

ACKNOWLEDGMENTS

I had only the pleasant task of presenting this work though the credit for the work on mass spectrometry on the quinazoline and ipecac alkaloids discussed herein goes to Dr. H. Budzikiewicz, my illustrious collaborator now in Stanford, California, U. S. A. I am grateful to Dr. J. C. Ray, Director, Indian Institute for Biochemistry & Experimental Medicine, Calcutta and to Prof. C. Djerassi of Stanford University for their kind interest in our work.

Last, but not the least, I must express my gratitude to the organizers of this symposium for kindly giving me an opportunity to talk about a subject in which the plant chemists are vitally interested.

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